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FOAM CELL PLAQUES IN THE INTIMA OF IRRADIATED SMALL ARTERIES (ONE HUNDRED TO FIVE HUNDRED MICRONS IN EXTERNAL DIAMETER)

JOHN F. SHEEHAN, M.D.

CHICAGO

The vascular changes produced in human patients as well as in experimental animals by roentgen radiation or the gamma rays of radium have been described in numerous original papers and reviews.[1] Some of these publications have been devoted primarily to the recording of vascular changes; others, to the reporting of the effect of radiation on various tissues and organs, the blood vessels of which have often shown changes sufficiently striking to be considered contributory to, or even responsible for, the other changes noted.

A fairly comprehensive survey of this literature revealed only one probable reference to the lesions to be described in this paper—foam cell plaques in the intima of small arteries. This sole reference consisted of an illustration with its accompanying legend in an article on radiobiology and radiopathology by Frances Carter Wood.[2] There was no elaboration or discussion of the lesion in the text. No mention was made of foam cells, but the illustration revealed a structure apparently identical with a foam cell plaque. Wood used the term "xanthomatous endothelial proliferation" to describe the pathologic change.

From the Department of Pathology of Loyola University School of Medicine and the Mercy Hospital Institute of Radiation Therapy.

1. (*a*) Warren, S.: Arch. Path. **34:** 443, 562, 749, 917 and 1070, 1942; **35:**121 and 304, 1943. (*b*) Warren, S. L.: The Physiological Effects of Radiation upon Organ and Body Systems, in Duggar, B. M.: Biological Effects of Radiation, New York, McGraw-Hill Book Company, Inc., 1936, vol. 1. (*c*) Borak, J.: Radiology **38:**481, 607 and 718, 1942. (*d*) Flaskamp, W.: Ueber Röntgenschäden und Schäden durch radioactive Substanzen, in Meyer, H.: Sonderbände zur Strahlentherapie, Berlin, Urban & Schwarzenberg, 1930, vol. 12. (*e*) Ellinger, F.: Die biologischen Grundlagen der Strahlentherapie, in Meyer, H.: Sonderbände zur Strahlentherapie, Berlin, Urban & Schwarzenberg, 1935, vol. 20: The Biologic Fundamentals of Radiation Therapy, translated by R. Gross, New York, Elsevier Publishing Company, Inc., 1941.

2. Wood, F. C.: Radiobiology and Radiopathology, in Pohle, E. A.: Theoretical Principles of Roentgen Therapy, Philadelphia, Lea & Febiger, 1938, fig. 127, p. 225.

PATHOLOGIC MATERIAL CONTAINING THE FOAM CELL PLAQUES

The foam cell plaques to be described here were observed in 8 specimens excised at varying intervals of time after the completion of courses of radiation therapy. Among these specimens were included 1 adenocarcinomatous rectum treated by separate courses of roentgen and radium therapy; 4 uteri from which adenocarcinoma of the endometrium had been eradicated by combined roentgen and radium therapy; 1 uterus with adenocarcinoma of the endometrium in which the neoplasm had not been destroyed by combined roentgen and radium therapy; 1 uterus with adenocarcinoma of the endometrium treated by radium therapy only; and finally 1 uterus with adenoacanthoma of the body which had been subjected to roentgen radiation only.

Paraffin sections were prepared from blocks of tissue fixed in 4 per cent solution of formaldehyde or Bouin's fixative. Representative sections from each block were stained with hematoxylin and eosin. Verhoeff's stain for elastic tissue and Masson's trichrome stain were used when indicated. No special staining for lipoid substances was possible since only paraffin blocks of the tissues studied were available.

A summary of the more important facts relating to these specimens is given in the accompanying table. More detailed information follows.

Specimen 1.—This rectum was excised about one month after the second of two intracavitary applications of radium one month apart. A 2 mm. brass filter and a 5 mm. rubber filter were used. The dose at each application was 1,200 milligram hours. The radium therapy had been instituted one month after the completion of a course of roentgen therapy in which the total dose was 5,000 roentgens (r), divided into 20 doses and delivered over a period of one month. Further details about the roentgen treatment are given in a previous paper.[3]

Examination of the specimen revealed an adenocarcinomatous ulcer a few centimeters proximal to the anal canal. This ulcer almost completely encircled the lumen. A few centimeters proximal to the carcinomatous ulcer, separated from it by grossly normal mucosa, was a band of bright red mucosa, 1.5 cm. wide, completely encircling the lumen. The red mucosa was not appreciably swollen. It was smooth except for scattered, irregular, flat-topped, slightly raised, dark green sloughs which covered superficial ulcerations. The largest erosion measured 7 by 7 mm. in area and was 1 mm. or less in depth. There was no gross thickening or induration of the rectal wall beneath the red mucosal band. The overlying serosa was bright

3. Schmitz, H. E.; Sheehan, J. F., and Towne, J.: Am. J. Obst. & Gynec. **45:**377, 1943.

red and smooth. The red mucosal band was considered to represent an area of proctitis induced by radiation.

Microscopic examination of a portion of the rectal wall which included the margins and the base of a representative radiation ulcer revealed in the deepest part of the ulcer loss of only the most superficial portion of the submucosa. In the mucosa in the margins of the ulcer were noted atrophic glands, dilated capillaries, swollen hyalinized muscle cells of the muscularis mucosae and rather massive infiltration by plasma cells. Poorly formed, partially hyalinized granulation tissue, infiltrated by plasma cells and marked by swollen bizarre-shaped fibroblasts and endothelial cells, occupied the portion of the submucosa immediately adjacent. The ulcer was covered by debris and a thin layer of fibrin and neutrophils, superficial to which in areas lay a rather thick layer of hyaline material, probably altered blood.

The ulcer base rested on an edematous portion of submucosa devoid of granulation tissue, in the upper reaches of which vascular changes were noted. Small arteries touching the necrotic debris filling the ulcer often showed fibrinoid necrosis and occlusion by hyaline thrombi. In a few arteries, 150 to 250 microns in external diameter, there were focal eccentric plaque-like thickenings of the intima with varying degrees of occlusion of the lumen. Accumulations of foam cells and hyaline material (altered fibrin or fused red cells) were responsible for the intimal thickenings. In small arteries close to the necrotic base of the ulcer neutrophils were added to the other elements in the thickened intima. The media of some of these arteries was normal; the media of others had undergone varying degrees of hyaline change (necrosis). The nuclei of the muscle fibers were swollen but still present in some arteries; they were entirely absent from others. Vacuoles were noted in the hyalinized media in areas. There was no cellular infiltration of the altered media or adjacent adventitia of most of these small arteries.

In addition to these vascular changes, in the edematous portions of the submucosa swelling of endothelial cells in capillaries and focal plasma cell infiltration were noted. The muscle fibers in the inner layer of the muscular coat of the rectum were somewhat atrophic. The entire muscular coat was somewhat edematous, particularly in its innermost portion. There was mild infiltration of the connective tissue between the two layers by lymphocytes, plasma cells and eosinophils. In the perirectal fat were encountered a few lymphocytes and eosinophils and a few small arteries (150 to 200 microns in external diameter) which showed changes similar to those noted in arteries of a similar size in the submucosa, namely, irregularly distributed deposits of hyaline material and foam cells in the intima with focal intimal thickenings and hyaline necrosis of parts of the media with or without neutrophilic infiltration.

Summary of Observations on Specimens

Specimen No.	Organ	Pathologic Diagnosis	Type of Radiation Therapy and Dose	Time Elapsed Between Completion of Radiation Therapy and Surgical Excision	Gross Evidence of Changes Induced by Radiation; Other Pertinent Gross Changes	Location of Arteries with Foam Cells in Intima
1	Rectum	Adenocarcinoma	Intracavitary application of radium, 2,400 mg.-hr.; filter, 2 mm. brass and 5 mm. rubber. Roentgen radiation, 5,000 r	1 month	In mucosa proximal to carcinoma, red mucosal band with shallow ulcers covered by green sloughs (proctitis due to radiation)	Submucosa near ulcer base; perirectal fat
2 to 5	Four uteri	Adenocarcinoma of the endometrium (grades 2 or 3)	Intrauterine application of radium, 6,000 mg.-hr.; filter, 2 mm. brass; Y tube in 3 cases, straight tube in 1. Roentgen radiation, 4,000 r to midpelvis	3-8 months	Areas of necrosis involving endometrium and adjacent myometrium at level of internal os, due to radiation; no carcinoma	Most numerous in myometrium or cervix near areas of necrosis; fewer at a distance, in myometrium and cervix
6	Uterus	Adenocarcinoma of the endometrium (grade 3)	Intrauterine application of radium, 6,000 mg.-hr.; filter, 2 mm. brass; Y tube. Roentgen radiation, 4,000 r to midpelvis	19 months	No gross changes due to radiation; extensive carcinoma in uterus, but not in adnexa	Myometrium near invading cancer; parametrium
7	Uterus	Adenocarcinoma of the endometrium (grade 2)	Intrauterine application of radium, 1,800 mg.-hr.; filter, 2 mm. brass; straight tube. No roentgen radiation	1 month	Area of radionecrosis involving endometrium and adjacent myometrium at level of internal os; carcinoma still presesnt	Myometrium near area of necrosis at internal os
8	Uterus	Adenoacanthoma of the endometrium (grade 3)	Roentgen radiation, 5,500 r. No radium	2 months	No gross changes induced by radiation; extensive carcinoma of uterine body; metastases to anterior lip of cervix and to ovary	Myometrium near invading cancer near internal os; ovary

Specimens 2 to 5.—These consisted of 4 uteri which had been objects of a previous study, some features of which have already been reported.[3] In table 1 of a previous paper [3] these uteri are listed under the initials D. C., M. S., P. G. and R. B., respectively. Each presented adenocarcinoma of the endometrium (grade 2 or grade 3) and was subjected to intensive combined radium and roentgen therapy—6,000 milligram hours of intrauterine application of radium and 4,000 r directed to the midpelvis. The uteri were excised three to eight months after completion of the radiation therapy. Pathologic examination of these uteri revealed no carcinoma, although a thorough search was made. Since a detailed report of the gross and microscopic observations on these uteri will be made in a separate communication, only those which are pertinent to the aim of this paper will be recorded here.

Gross examination of these uteri revealed in each at the level of the internal os an area of radionecrosis which involved the endometrium and the inner portion of the adjacent myometrium. Microscopic examination

disclosed marked vascular changes in the viable tissues adjoining the areas of necrosis. These changes consisted predominantly of swelling of the endothelial cells with or without elevation of portions of the endothelium and accumulation beneath the elevated endothelium of fibrin, hyaline material (old fibrin or fused red cells), fluid and cells in various combinations. The larger plaques thus formed almost always contained fibrin or hyaline material, the latter concentrated as a bright red band immediately beneath the endothelium or dispersed more diffusely throughout the thickened intima as a paler red structureless substance, sometimes vacuolated. Cells, particularly foam cells or red cells, were intermixed with the more diffusely distributed type of hyaline material or were located deep to the more concentrated form. In some plaques masses of red cells with but little hyaline material and with few or no foam cells were present; in others (much more numerous) foam cells predominated, at times being the only apparent constituents of the plaques. However, often other cells mingled with the foam cells to form a variety of combinations. These cells were red cells, lymphocytes, monocytes, eosinophils and more rarely neutrophils or even plasma cells.

The plaques were irregularly distributed. In some preparations longitudinal sections of certain arteries with long axes perpendicular to the areas of necrosis were obtained. In some of these vessels, as the areas of necrosis were approached there was a transition from plaques composed predominantly of foam cells to those made up of red cells or of red cells and hyaline material. In some arteries, fairly large plaques composed of foam cells were found in one part of the artery and smaller plaques containing fluid and a few cells, notably lymphocytes or lymphocytes and monocytes, elsewhere. A few eosinophils were commonly present. At times in the peripheral parts of the areas of necrosis foam cells could be distinguished in arteries that were undergoing complete dissolution or which were apparently thrombosed. The internal elastic membrane, the media and the adventitia of the arteries which contained these foam cell plaques were often normal. There was no intimal fibrosis associated with the plaques, and only rarely thrombosis. Although the foam cell plaques were most numerous near the gross areas of radionecrosis, they were also found at some distance away in parts of the myometrium which showed no other deviations from the normal which might be attributed to radiation. One plaque was found in an artery in a tumor diagnosed as leiomyoma.

The foam cell plaques varied considerably in thickness. They were often several times thicker than the media and caused marked narrowing or even obliteration of the lumen. When much hyaline substance was present along with the foam cells, a false impression of thrombosis often arose. True thrombosis was sometimes seen in the vicinity of these plaques.

The arteries which contained the foam cell plaques varied from 100 to about 500 microns in external diameter. In the smaller of these vessels (100 to 300 microns) those parts of media adjacent to plaques were at times necrotic; other portions of the media were also at times necrotic, even some at a distance from plaques. If necrosis occurred, the normal muscle and fibrous tissues of the media were replaced by a hyaline nonnucleated material, which took a brighter red stain in hematoxylin-eosin sections than normal smooth muscle did. Often no cellular infiltration of the altered media occurred; the internal elastic membrane often persisted intact, and the adventitia seemed normal. In other instances, however, the necrotic media was infiltrated by a few neutrophils; the internal elastic membrane failed to show its characteristic staining reaction (Verhoeff's stain), and the adventitia was edematous and infiltrated by a few lymphocytes, monocytes, eosinophils and even a few neutrophils. Sometimes the necrotic media was thicker than the normal media, and the lumen was narrowed or obliterated, but thrombosis did not supervene. Often normal small arteries lay alongside necrotic arteries of a similar size. Such completely necrotic small arteries were encountered in the cervix in many of these uteri, often in parts of the fibromuscular coat which showed no other radiation-induced changes. Small irregular nonnucleated hyaline masses, partly vacuolated, with surrounding narrow zones of edematous connective tissue infiltrated by a few chronic inflammatory cells were sometimes encountered. These structures may represent stages in the final dissolution of necrotic small arteries.

Other changes were noted in the small arteries near the gross areas of necrosis. Some of these could be considered effects of radiation, particularly intimal thickening by hyaline acellular connective tissue, probably swollen collagen. This change was frequently seen. Less common were fibroblastic intimal thickening, true thrombosis with partial organization of some of the thrombi and deposition of hemosiderin and, most uncommon of all, deposition of hemosiderin in intimas thickened by hyaline material.

Vascular changes not attributable to radiation occurred in some of the uteri and for a time caused confusion as to their interpretation. Chief among these were the various stages of arterial change described as more or less characteristic of subinvolution of the uterus, in particular the "vitreous" change which the swollen internal elastic lamina was undergoing and the formation of new blood vessels within the persisting swollen elastic tissue representing the remains of old blood vessels. In the uteri just described the arteries with foam cell plaques possessed normal internal elastic membrane as long as the media remained intact. If the media underwent necrosis, the internal elastic membrane disappeared. There was never any accumulation of elastica in the adventitia. After some experience it was possbile to separate the vascular changes of subinvolution from those due to radiation.

In the 4 uteri just described a particularly excellent opportunity was afforded for study of the distribution of the foam cell lesions and of the other vascular changes throughout the uterine wall. The distribution of the lesions with reference to the gross areas of radionecrosis could also be determined. Numerous blocks from all parts of the uteri were available. The method of obtaining the blocks has been discussed in a previous paper.[3]

Specimen 6.—This uterus, like the 4 just described, was subjected to intensive irradiation—6,000 milligram hours of intracavitary application of radium, and 4,000 r directed to the midpelvis. The uterus was excised nineteen months after the completion of the radiation therapy. A deeply invading and widely extending tumor of the endometrium, diagnosed as adenocarcinoma, was still present. The cervix was involved from above, but there were no metastases in the tubes or the ovaries, which were excised simultaneously. The uterine cavity was dilated by a submucous tumor, diagnosed as leiomyoma, of respectable size—5.2 by 4 by 3.4 cm. Only routine sections of the uterus, tubes and ovaries were made. They revealed intimal foam cells in arteries in the parametrial tissues as well as in the myometrium adjacent to the malignant tumor. In the parametrium one artery between 450 and 500 microns in external diameter showed foam cells, fibrin and a few neutrophils beneath a focally elevated endothelium; in another

artery fibrin and foam cells only were observed. The internal elastic membrane, the media and the adventitia were essentially normal. In the myometrium arteries ranging in size from 150 to 275 microns showed intimal plaques of varying sizes and composition. Some were composed of foam cells alone; others, of foam cells, fibrin and lymphocytes, and still others, smaller in size, of lymphocytes and fluid. The elevated overlying endothelial cells were swollen. In a few small arteries thick intima, composed of hyaline material, possibly altered collagen, was noted. Some of these arteries were occluded. The altered arteries in the myometrium were not near frank areas of necrosis, although some were near large areas of hyalinization of the myometrium.

Specimen 7.—In specimens 2 to 5, inclusive, gross areas of radionecrosis at the internal os were noted. A similar area at the internal os was observed in this uterus, excised about one month after the completion of the radiation therapy. The therapy consisted of the intrauterine use of radium. The total dose amounted to only about 1,800 milligram hours. A tumor, diagnosed as adenocarcinoma, almost completely limited to the portions of endometrium still undestroyed by radioactivity, was encountered. The most pronounced vascular changes were noted in the inner layer of the myometrium near the gross area of radionecrosis. In one small artery, 170 to 200 microns in external diameter, swollen endothelial cells, elevated in areas, were noted. Beneath the elevated cells, between them and the internal elastic membrane, foam cells, a few lymphocytes, a rare neutrophil and masses of hyaline material were identified. In another plaque hyaline material, red cell shadows, a few lymphocytes and neutrophils were seen. In still another, fibrin and monocytes predominated. The internal elastic membrane, the media and the adventitia seemed normal. In the hyalinized myometrium near the large area of necrosis a few small arteries about 170 microns in external diameter had swollen hyalinized intima. Other arteries of a similar size showed fibrinoid necrosis. These merged with the necrotic tissues lining the uterus.

Specimen 8.—This uterus was excised two months after a course of treatment with external radiation only. A total dose of 5,500 r was administered but over a period of six months or more rather than over the customary four week period. Examination of the uterus and adnexa revealed an ulcerating tumor of the lower part of the uterine body involving the internal os and the upper portion of the endocervix, diagnosed as adenoacanthoma. The tumor had metastasized to the anterior lip of the cervix and to one ovary. This ovary contained a tumor nodule 5 mm. in diameter. Otherwise the ovaries showed no change except atrophy. There were no gross areas of radionecrosis. Microscopic examination of routine sections revealed vascular changes in numerous arteries in the ovaries and in one or two arteries in the myometrium near the internal os but not in other portions of the myometrium or in the cervix. The few altered myometrial arteries measured about 350 microns in external diameter. In the intima eccentric plaquelike accumulations of foam cells with a few lymphocytes intermixed caused considerable narrowing of the lumens of arteries. These arteries were not located near areas of necrosis.

The most pronounced vascular changes were observed in the ovaries. In addition to the usual obliterative changes seen in the arteries of atrophic ovaries, other changes most probably attributable to radiation were noted. Well defined accumulations of foam cells were present in the intima of arteries varying from 135 to 450 microns in external diameter. In some of these a few lymphocytes and neutrophils were intermixed. The lumens were often markedly narrowed. The lumens of two small arteries (250 and 450 microns in external diameter, respectively) were filled with foam cells and debris, in which angular slitlike spaces, apparently once occupied by cholesterol crystals, could be seen. The internal elastic membrane, the media and the adventitia were normal. The lumen of one artery, about 400 microns in external diameter, was obliterated by a hyaline thrombus. The wall of this artery was normal. Although foam cells could be seen in the intima of numerous small arteries in the ovaries, it is to be noted that cholesterol crystals were identified only in what seemed to be the lumens of vessels, despite the fact that they were accompanied by foam cells and despite the fact that adjacent small arteries contained foam cells in the intima.

SUMMARY OF THE MORE IMPORTANT PROPERTIES AND RELATIONS OF THE FOAM CELL PLAQUES

Nature and Composition of the Plaques.—The lesions emphasized in this paper are essentially eccentrically situated plaquelike thickenings of parts of the intima of small arteries. They contain foam cells alone or foam cells intermixed with fibrin, hyaline material (fused red cells?), fluid, red blood cells, lymphocytes, monocytes, eosinophils and neutrophils in various combinations; rarely cholesterol crystals are observed (figs. 1, 2 and 3).

The foam cells often occur alone; commonly they are observed with hyaline material (probably fused red cells), fibrin or red cells (fig. 5). Lymphocytes, monocytes and eosinophils when accompanying the foam cells are usually few.

The foam cells in a plaque may be few or numerous. As many as thirty have been observed in one plaque.

In the intimal plaques there is no fibroblastic proliferation (figs. 1 and 5).

Location of Plaques in Arterial Wall; Condition of the Endothelium.—The foam cells and other constituents of the plaque in the intima are located between the elevated endothelium and the internal elastic membrane (figs. 1 and 5). The endothelial cells over the plaque are usually enlarged. Both the cytoplasm and the nuclei are swollen. The large endothelial cells often project into the lumen of the involved vessel.

Condition of the Internal Elastic Membrane, Media and Adventitia Adjacent to the Lesion.—There is often no change in the internal elastic membrane, the media or the adventitia adjacent to a foam cell plaque (fig. 5). However, particularly in arteries 100 to 300 microns in external diameter, the media may undergo hyaline change with disappearance of the nuclei of muscle cells and fibroblasts, with or without neutrophilic infiltration (fig. 7). If the media becomes

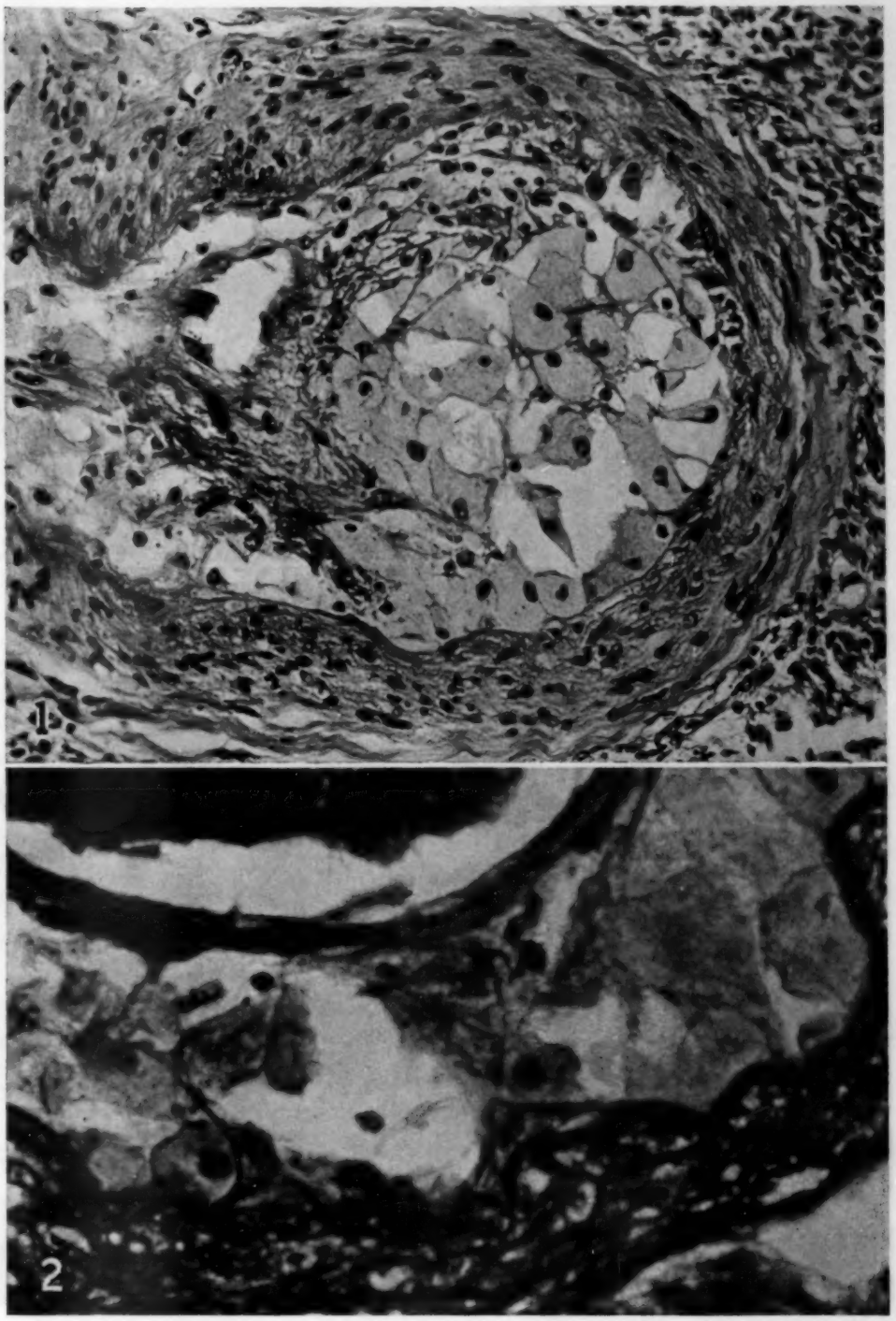


Fig. 1.—Typical foam cell plaque in the intima of a small artery (about 375 microns in external diameter) in the myometrium, producing marked narrowing of the lumen. The lumen, surrounded by about four, widely separated, swollen, dark endothelial cells, is located slightly above and to the left of the center of the field. A few lymphocytes and some fibrin are seen with the foam cells. The media is essentially normal. × 310.

Fig. 2.—Foam cell plaque in a small myometrial artery (135 to 170 microns in external diameter). A part of the wall under high magnification is seen. Foam cells only are present. They are situated between normal wavy internal elastic membrane (below and to the right) and the lumen (above and to the left). Parallel to the lumen, next to the endothelium, is a thin layer of fibrin. Verhoeff's elastic tissue stain. × 600.

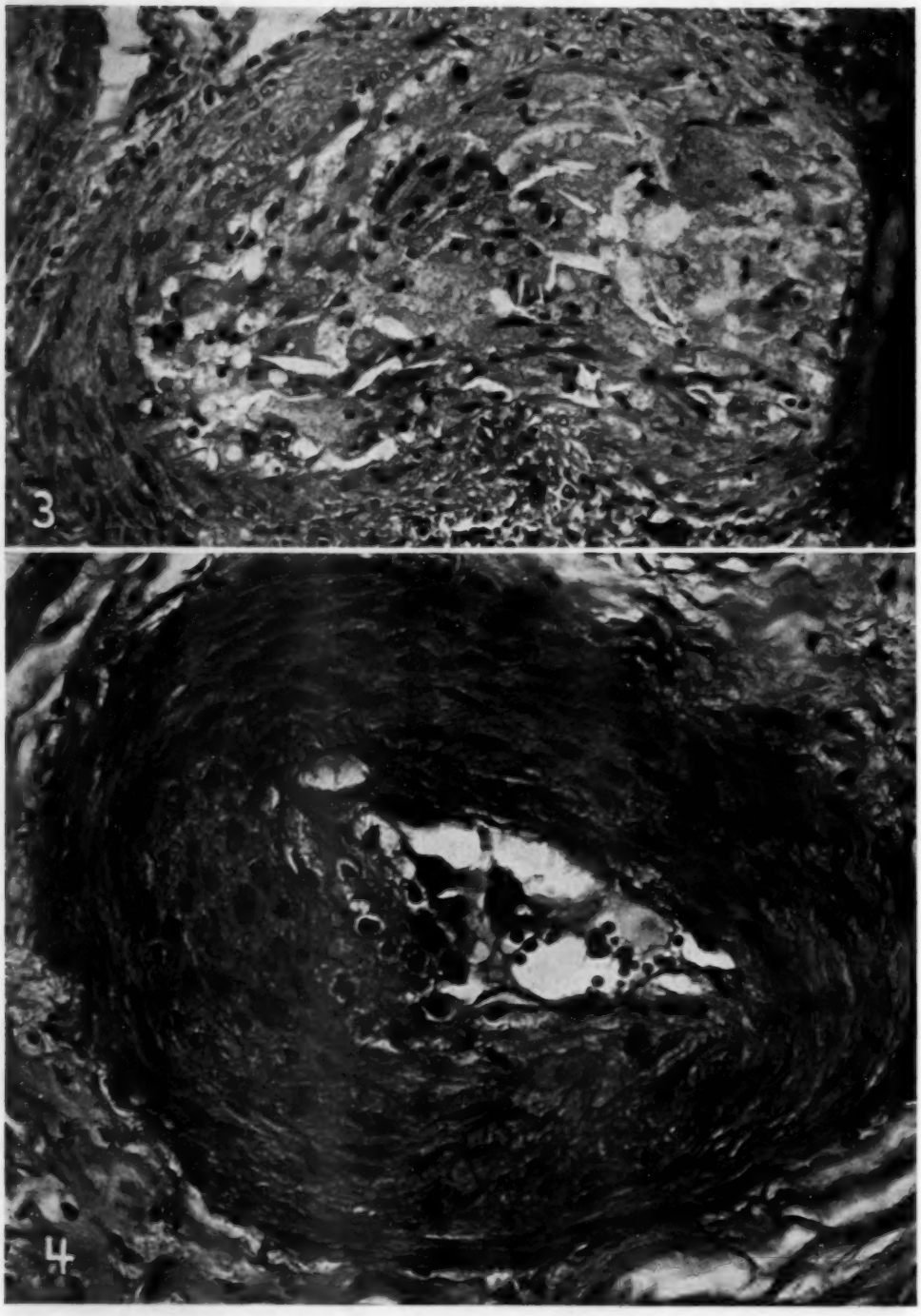


Fig. 3.—Small artery in an ovary, the lumen occluded by debris, cholesterol crystals and foam cells. The media is essentially normal. The black smudge covering the right lateral portion of the media is caused by a fold in the section. × 310.

Fig. 4.—Early plaque in a small artery. Markedly swollen dark endothelial cells (four or five in number) surround the extremely small lumen. The endothelial cell on the right is elevated and markedly displaced to the left. Between it and the media are vacuoles (fluid), a few monocytes (large dark round dots), a few lymphocytes (small dark round dots) and red cells (small pale round dots). × 385.

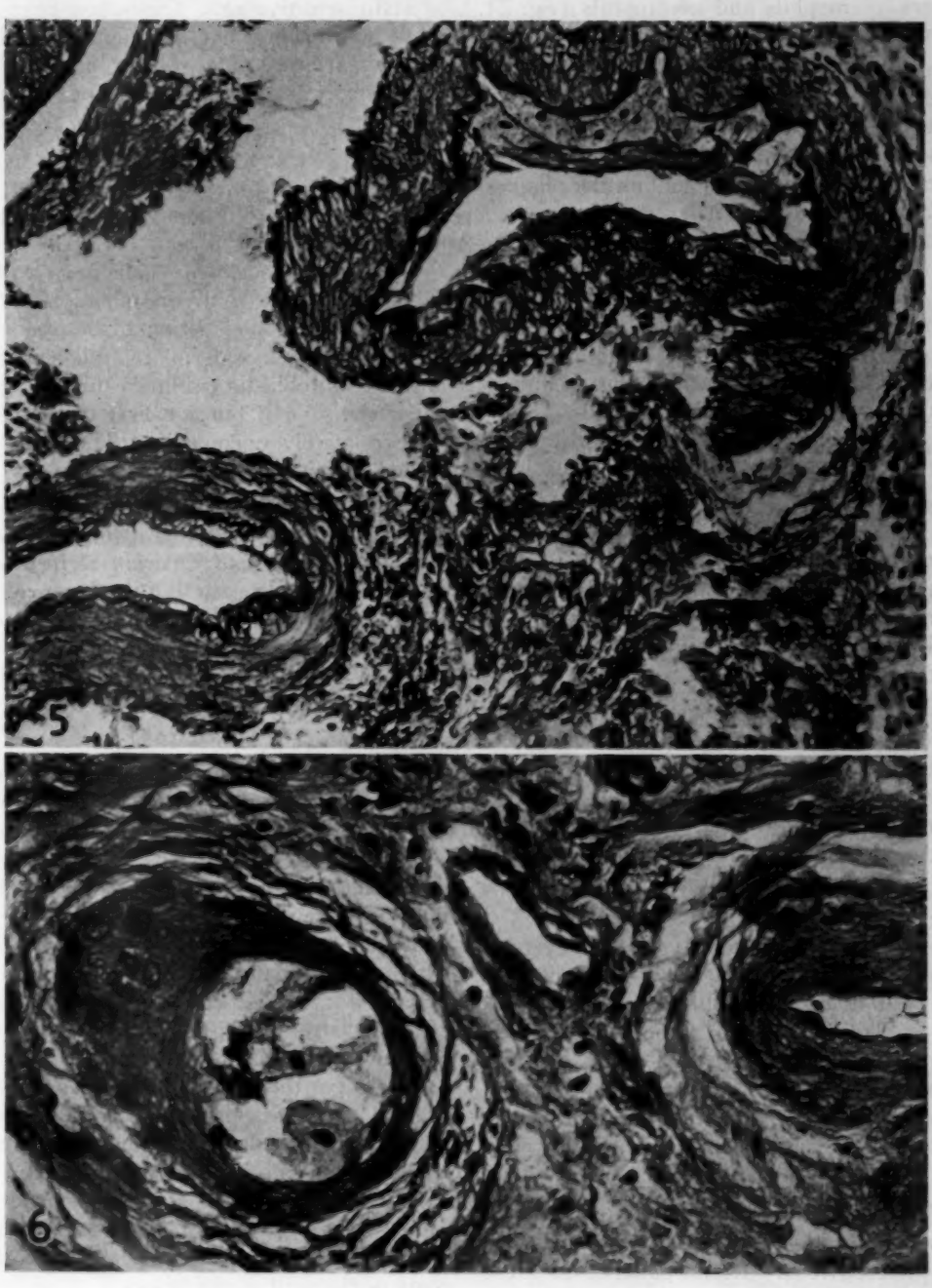


Fig. 5.—Two small arteries (about 135 to 170 microns in external diameter). Note the foam cell plaque in the artery in the right upper part of the field and the eccentric intimal thickening. Fibrin is present in the plaque, near the lumen. The internal elastic membrane and the media are normal. There is an elevation of portions of the endothelium of the artery in the left lower part of field, caused by fluid only. Verhoeff's elastic tissue stain. × 111.

Fig. 6.—Arteriole (about 100 microns in external diameter) from the cervix uteri. No lumen is present. The foam cells fuse with necrotic hyalinized portions of the media. × 375.

necrotic (hyaline change), the internal elastic membrane may disappear and the adventitia may show edema and mild infiltration by lymphocytes, monocytes, eosinophils and neutrophils (fig. 7). Sometimes red cells are present in the adventitia.

Size of Artery Involved; Location of Lesions Along Course of Vessel.—The foam cell plaques are observed in small arteries, about 100 to 500 microns in external diameter. In any one irradiated area only a few arteries contain the plaques. The plaques are distributed irregularly even along the course of a single artery. Transitions from foam cell plaques to plaques containing hyaline material (probably fused red cells) (fig. 8) or masses of red cells are seen, as well as transitions from small plaques containing only lymphocytes, monocytes and fluid (figs. 4 and 5) to larger ones containing the foam cells.

Effect of Lesion on Caliber of Lumen.—The foam cell plaque may be two or three times as thick as the media of the involved artery and often may cause marked narrowing or even obliteration of the lumen (fig. 1). It is only rarely associated with thrombosis, although the occurrence of much fibrin or hyaline material in the thick occluding plaques gives a false impression of thrombosis.

Relation of Lesions to Areas of Necrosis Due to Radiation.—The foam cell plaques are most numerous in arteries near areas of necrosis due to radiation (as in the uterus) or of ulceration due to radiation (as in the rectum). However, they occur also at a distance from such areas of necrosis or ulceration, as in the perirectal fat, the parametrium and the ovaries.

Relation of Lesions to Type of Radiation Therapy; Time of Appearance.—The plaques are observed in cases in which the patient was treated by radium only or roentgen radiation alone or by both combined.

The plaques appear as early as one month and are present as late as nineteen months after the completion of the radiation therapy.

Ultimate Fate of Lesions.—The ultimate fate of the plaques, at least in the larger of the small arteries (300 to 500 microns in external diameter), is not obvious. In the smaller of these arteries (100 to 300 microns) foam cell plaques adjacent to the necrotic portions of the media finally seem to fuse with the adjacent hyalinized media to form unorganized vacuolated hyaline masses of irregular outline, which gradually decrease in size and probably ultimately disappear, possibly as a result of the inflammatory reaction in the adjacent adventitia (fig. 6).

Localization of Lesions to Arteries.—Foam cell plaques were not observed in any vessel definitely identifiable as a vein. Arteries with necrotic media may lose their internal elastic membrane (or at least the membrane may fail to stain selectively). They then may be confused with veins, but a comparison with nonnecrotic arteries of a similar size in the same field usually facilitates the differentiation.

Relation of Lesions to Other Radiation-Induced Arterial Changes.—Other arterial changes that have been described at various times as effects of radiation were seen in this material: hyaline fibrous intimal thickening (swelling of collagen?), sometimes with occlusion; thrombosis with organization of thrombi with or without deposition of hemosiderin; deposition of hemosiderin in thickened hyaline intima; fibroblastic intimal thickening; acute panarteritis. All but the first of these changes were relatively uncommon. There was no observed relation between these changes and the formation or the location of foam cell plaques.

Relation of Lesions to Involutionary Changes Seen in Uterine and Ovarian Arteries.—There was no observed relation of the foam cell plaques to any of the various vascular changes commonly encountered in the uteri and ovaries of aging or aged women, particularly those who have borne children.

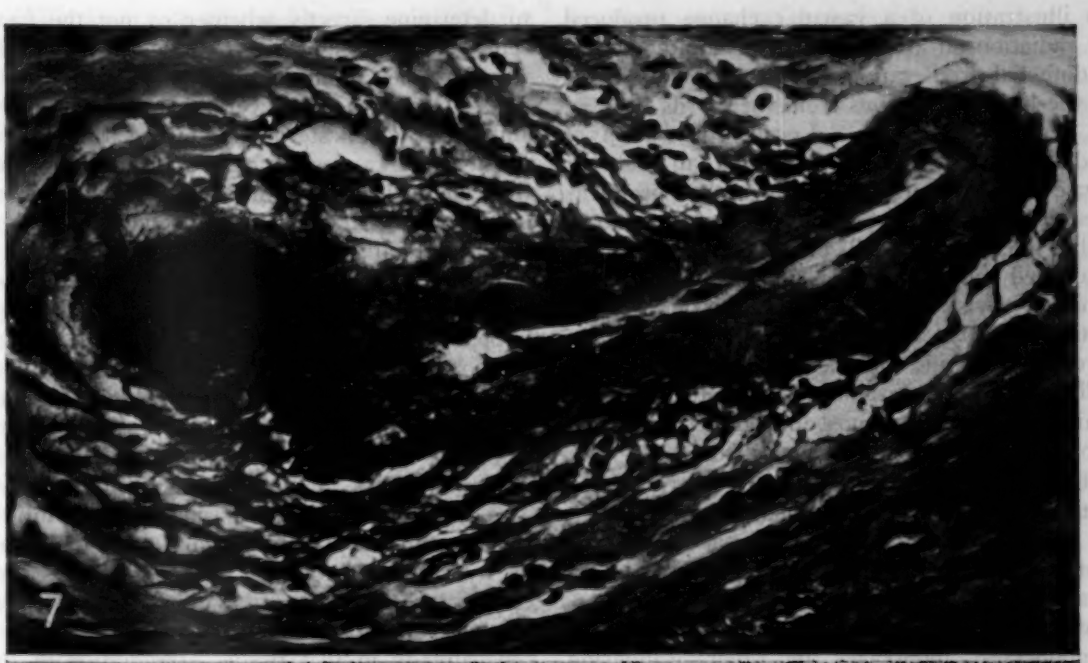
COMMENT

The genesis of the foam cell lesion cannot be definitely determined from the data recorded here. Nevertheless a theory may be formulated. Before this is done, however, certain facts must be recalled. First, the intima of arteries less than 300 microns in diameter consists only of endothelial cells and internal elastic membrane.[4a] Second, the foam cells in the arterial lesion described in this paper are confined to the intima of small arteries most of which are less than 300 microns in diameter. Hence two main possibilities seem to present themselves: The foam cell plaque results either from local proliferation of endothelial cells and simultaneous or subsequent transformation of these into foam cells or from focal accumulation in the intima of cells (foam cells or precursors) which have migrated into the intima from the lumen or from the outer coats of the wall of the vessel.

Hueper [5] was of the opinion that proliferation of endothelial cells gave rise to the foam cells observed in the intima of arteries of animals which had received injections of pectin. It has already been remarked that Wood [2] probably referred to the foam cell lesion when he used as

4. Maximow, A., and Bloom, W.: A Textbook of Histology, ed. 4, Philadelphia, W. B. Saunders Company, 1942, (*a*) p. 245; (*b*) p. 112.

5. Hueper, W. C.: Arch. Path. **34**:883, 1942.



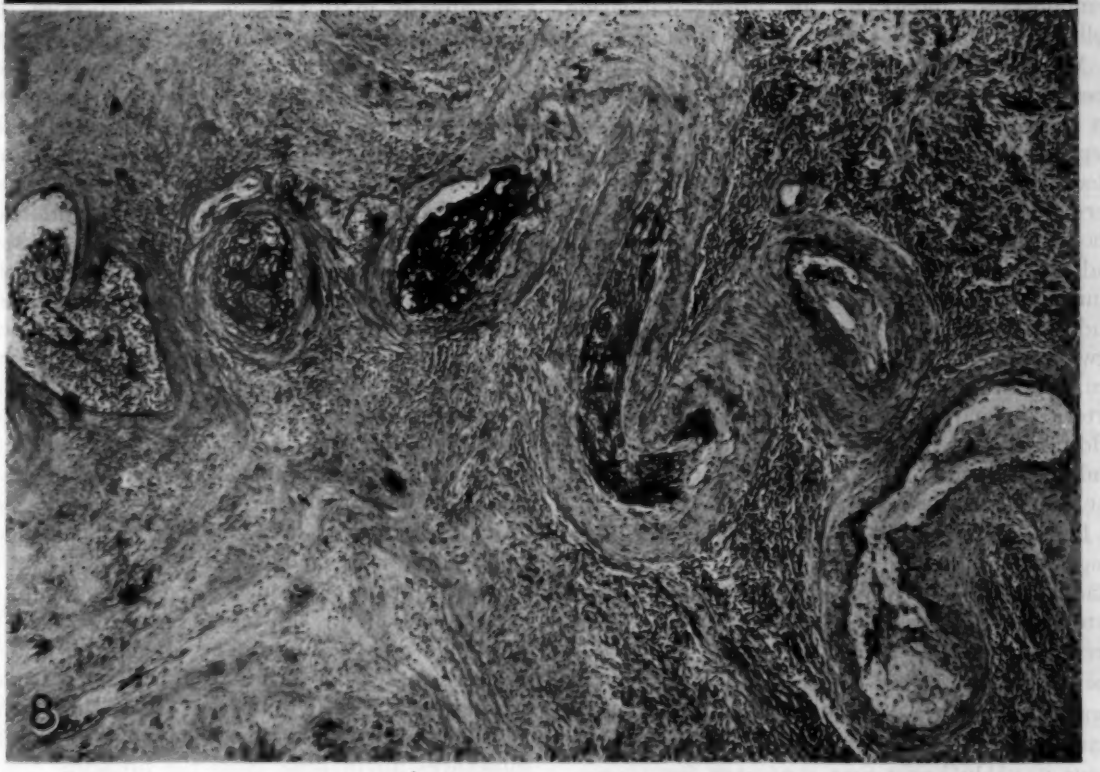


Fig. 7.—Small artery (about 135 microns) from the cervix uteri. The media of the left half of the vessel is completely necrotic and hyalinized, appearing bright red in a section stained with hematoxylin and eosin. The collagen fibers in the adventitia are separated (edema); the adventitia is infiltrated by lymphocytes, monocytes, a few eosinophils and fewer neutrophils. × 385.

Fig. 8.—Small artery (about 375 microns) cut in various planes. Observe the transition from a completely necrotic portion of the artery in an area of radionecrosis occupying the left half of the field to a viable portion of the artery with a foam cell plaque in the living myometrium in the right half of the field. Thrombosed portions of artery (with large dark masses in the lumen) connect the other two portions. × 75. The foam cell plaque in the portion of the artery in the extreme right part of the field is shown at a higher magnification in figure 1.

an illustration of a vascular change produced by radiation an arteriole showing xanthomatous endothelial proliferation. His view of the genesis of the lesion is obvious from the words he used to describe the lesion. Local proliferation of endothelial cells, however, seems an unlikely cause of the foam cell lesions in the specimens described in this paper. No mitoses were noted either in endothelial cells or in foam cells. Multinucleated endothelial cells, possibly indicative of amitotic division, were found occasionally but not constantly. Finally, the deeply staining, swollen basophilic endothelial cells over the foam cell plaques were a strong contrast to the palely staining foam cells, almost immediately adjacent. No transition forms were identified.

Cellular migration into the intima from the external coats of the vessel wall seems ruled out by the observation that in a great number of instances the media and the adventitia adjacent to a foam cell plaque, even a large one, were normal and were not infiltrated by foam cells, lymphocytes, monocytes or other wandering cells.

Migration of cells from the arterial lumen seems the most logical of all the possibilities. In favor of this view is the well known increased permeability of endothelial cells of irradiated blood vessels (Borak [1e]). In the specimens described here the endothelial cells over the plaques showed the cytoplasmic and nuclear swelling indicative of radiation effect and can be presumed to have been more permeable than normal endothelial cells. The presence of fluid and a few lymphocytes and eosinophils beneath swollen endothelial cells in arteries or portions of arteries which showed no other abnormality is additional evidence of increased permeability of endothelial cells and of migration of cells from the lumens of the vessels.

It does not seem likely that foam cells as such migrated from the blood stream. In small plaques foam cells were usually absent; lymphocytes or monocytes were the only cells constantly present. It seems probable that the latter were the migrating cells. Once in the intima the monocytes and the lymphocytes could have been transformed readily into foam cells, the lymphocytes possibly being first converted into monocytes (Maximow and Bloom; [4b] Nye and Parker [6]).

Speculation concerning the genesis of the plaques would rest on a firmer foundation if the chemical substances in the cytoplasm of the foam cells were known with certainty. During the present investigation there was no opportunity to determine directly whether or not the foam cells contained lipoid or doubly refractile substances, since paraffin blocks alone were available. The fact, however, that crystals, presumably of cholesterol, accompanied the foam cells in a few lesions warrants the inference that the foam cells contain cholesterol and are in reality xanthoma cells. Wood [2] considered them xanthomatous.

By the ingestion of lipoid substances, either proliferated endothelial cells or immigrated lymphocytes and monocytes could give rise to the foam cells of the foam cell intimal lesion. The arguments already advanced, however, definitely favor one of these alternatives—the phagocytosis of lipoid substances, probably cholesterol, by lymphocytes and monocytes in the intima. The occurrence of lipoid substances in the intima remains to be explained. Again two possibilities present themselves. The lipoid substances may enter the intima from the blood stream or be produced in the intima by the disintegration of cells already in that tissue. Irradiation of the vascular endothelium increases its permeability to such an extent that cells such as lymphocytes and red cells readily pass through it. The blood cholesterol or other lipoid substances should traverse it with even greater ease. On the other hand, the possibility of the lipoid substances arising in the intima itself during the dissolution of cells, particularly red cells, cannot be dismissed. The red cells are known to contain such substances. Winternitz and his associates [7] considered intramural vascular hemorrhage a possible forerunner of atheroma. Gross and Wolbach, [8] in trying to determine the origin of the lipoid substances in the xanthoma cells seen in sclerosing hemangioma, considered the possibility that the lipoid substances may have been those freed by the disintegration of red cells. They rejected this possibility on the ground that sufficient lipoid substances could not be furnished by the red cells in the angioma to make up the total quantity of lipoid substances contained in the xanthoma cells. The presence of red cells, often in masses, in the intima of irradiated small arteries, with or without accompanying foam cells, lends support to the view that the lipoid substances might arise from the breaking down of red cells. The almost constant presence in the foam cell plaques of hyaline material, probably altered blood clot or fused red cells, strengthens the possibility.

6. Nye, R. N., and Parker, F., Jr.: Am. J. Path. **6**: 381, 1930.

7. Winternitz, M. C.; Thomas, R. M., and Le Compte, P. M.: The Biology of Arteriosclerosis, Springfield, Ill., Charles C Thomas, Publisher, 1938, p. 35.

8. Gross, R. C., and Wolbach, S. B.: Am. J. Path. **19**: 533, 1943.

If the foam cells are really xanthoma cells, the possibility of a generalized disturbance of the metabolism of lipids must be considered. No studies were made of the blood lipids in the cases discussed here. Since none of the patients have died, there has been no opportunity to study the arteries in areas outside the field exposed to radiation. It would seem unlikely, however, that each of the 7 patients with carcinoma of the endometrium, selected at random for intensive radiation therapy, could be suffering from generalized lipidosis. Furthermore, no mention of radiation as a cause of generalized xanthomatosis or even of localized xanthoma is made in a recent monograph (Thannhauser [9]). While some of the cellular changes produced by radiation have been attributed to alterations in tissue lipids, such alterations are not constant. Indeed in a recent review of the effects of radiation on normal tissues, it is definitely stated that there is no clearcut effect of radiation on the blood cholesterol (Dunlap [10]). The possibility that the foam cell lesion of irradiated arteries is merely one manifestation of generalized xanthomatosis seems most improbable.

The evidence already presented in this discussion favors the following theory regarding the genesis of the foam cell plaque: Irradiation of endothelial cells causes not only morphologic changes but also sufficient change in their permeability to permit red cells, as well as lymphocytes and monocytes, to pass through them and to accumulate in the intima; the disintegration of red cells or of thrombi in the intima liberates lipoid substances, subsequently phagocytosed by monocytes, which are thus converted into foam cells. The possibility of a prior conversion of lymphocytes into monocytes is conceded, as well as the possibility that the foam cells arise through monocytic phagocytosis of blood lipids which penetrate the intima from the lumen.

One assumption has been made in the preceding paragraphs—that the foam cell plaques are the result of irradiation of the tissues involved. The fact that the plaques are most numerous where the radiation effects are most intense, near areas of radionecrosis, indicates that the plaques are produced directly or indirectly by radiation. Inflammation accompanying the necrosis produced by radiation is not responsible, at least not entirely, since the foam cell lesions are found in arteries at a distance from areas of necrosis or ulceration, as in the perirectal fat, the parametrium and the ovaries. Furthermore, the endothelial cells over the plaques usually show the swollen cytoplasm and nuclei which are fairly characteristic of radiation effect. Not uncommonly hyaline necrosis of the media, produced by radiation, is found adjacent to the plaques. These morphologic evidences of the effect of radiation on the cells and tissues immediately bordering on the plaques make plausible the assumption that the plaques themselves are manifestations of the effect of radiation.

The fact that the foam cell plaques occurred in an irradiated rectum as well as in uteri and ovaries rules out the possibility that the foam cell plaque is just one more of the various arterial changes noted in senile uteri and ovaries and in subinvoluted uteri.

Despite a vast literature on the changes induced by radiation, there exists a paucity of references to structures suggesting foam cells or xanthoma cells in irradiated arteries. The probable explanation is that the foam cells have been present, at least in some cases, but have been interpreted as vacuoles or as swollen vacuolated endothelial cells. Swelling and proliferation of endothelial cells have been observed and reported by numerous investigators (Gassmann; [11a] Thies; [12] Wolbach; [13] Petersen and Hellmann; [14] Dobrovolskaia-Zavadskaia; [15] Dyroff; [16] Schmidt; [17] Mottram; [18] Englmann; [19] Borak; [20] Warren [1a]). Vacuolation of endothelial cells was noted by Gassmann,[11a] Halkin,[21] Wolbach,[13] Dobrovolskaia-Zavadskaia,[15] Fahr,[22] Pullinger [23] and others. Gassmann's [11a] original illustrations of the intimal and medial changes in irradiated arterioles were considered sufficiently representative to be reproduced in

9. Thannhauser, S. J.: Lipidoses: Diseases of the Cellular Lipid Metabolism, New York, Oxford University Press, 1940.

10. Dunlap, C. E.: Arch. Path. **34**:562, 1942.

11. Gassmann, A.: (*a*) Fortschr. a. d. Geb. d. Röntgenstrahlen **2**:199, 1899; (*b*) Arch. f. Dermat. u. Syph. **70**:97, 1904.

12. Thies, A.: Mitt. a. d. Grenzgeb. d. Med. u. Chir. **14**:694, 1905.

13. Wolbach, S. B.: J. M. Research **21**:415, 1909.

14. Petersen, O. H., and Hellmann, J.: Strahlentherapie **11**:474, 1920.

15. Dobrovolskaia-Zavadskaia, N.: Lyon chir. **21**: 397, 1924.

16. Dyroff, R.: Arch. f. Gynäk. **136**:141, 1929.

17. Schmidt, H. R.: Ergebn. d. med. Strahlenforsch. **4**:325, 1930.

18. Mottram, J. C.: Brit. J. Radiol. **5**:643, 1932.

19. Englmann, K., in Holfelder, H.: Die Röntgentiefentherapie, Leipzig, George Thieme, 1938.

20. Borak,[1c] p. 607.

21. Halkin, H.: Arch. f. Dermat. u. Syph. **65**:201, 1903.

22. Fahr, T.: Virchows Arch. f. path. Anat. **254**: 277, 1925.

23. Pullinger, B. D.: J. Path. & Bact. **35**:527, 1932.

later works, even as recent as 1941.[24] In Gassmann's illustrations the intima is shown thickened by the proliferation of endothelial cells. Numerous vacuoles are present in the intima (and also in the media). Most of these are coarse vacuoles. Some are definitely inside cells, compressing and displacing the nuclei. According to Gassmann,[11a] some of the vacuoles are empty; others are traversed by an unstained, finely fibrillary reticular material. He did not describe any of the vacuolated intimal cells as foam cells despite the fact that he knew what foam cells were, since he reported their appearance in the connective tissue of the irradiated skin of a dog (Gassmann [11b]). In his report in 1904 he noted again arterial changes similar to those which he had reported in 1899. The intimal changes depicted by Gassmann do not resemble the foam cell plaques described in this paper.

Fat in irradiated endothelial cells has only rarely been reported (Obersteiner, cited by Colwell and Russ;[25] Alpers and Pancoast [26]). It is interesting that these papers issued from neurologic institutions. No mention of endothelial proliferation was made in the second of the two papers (Alpers and Pancoast [26]), the only one available. Besides intimal thickening due to endothelial proliferation, obliterative endarteritis and intimal collagen production have been observed (Baermann and Linser;[27] Wolbach;[13] Dobrovolskaia-Zavadskaia;[15] Montgomery [28]). Finally, medial changes similar to those described in this paper, namely, hyaline necrosis with or without neutrophilic infiltration or vacuolation, have been reported by several authors (Gassmann;[11a] Thies;[12] Wolbach;[13] Haendly;[29] Schmidt;[17] Windholtz [30]).

The foam cell plaques in irradiated arteries resemble early atheromatous plaques. In fact, a photomicrograph of a small pancreatic artery used by Leary [31] to illustrate an early lesion of atherosclerosis would serve equally well to depict some of the foam cell plaques induced by radiation. However close scrutiny, particularly of the condition of the adjacent endothelium, might disclose differences.

SUMMARY

An uncommon, or at least a rarely described, lesion of small arteries (100 to 500 microns in external diameter) has been observed in several irradiated organs. The lesion consists of a plaquelike thickening of the intima due to a collection of foam cells alone or of foam cells mixed with various other cells, fluid, fibrin or hyaline material, between the endothelium and the internal elastic membrane. Pathologic changes may be found in the adjacent internal elastic membrane, media and adventitia, but these structures are often normal. The plaque may cause marked narrowing or even occlusion of the lumen of the vessel. Thrombosis, fibroblastic proliferation of the intima or deposition of elastic tissue in the thickened intima seldom result.

These foam cell plaques have been found in the arteries of organs subjected to roentgen therapy only, radium therapy only or to both combined.

The plaques probably result from migration into the intima from the blood stream of lymphocytes and monocytes and subsequent transformation of these into foam cells by their ingestion of lipids which have been freed by the dissolution of red cells in the intima or which have accumulated in the intima after passage across portions of the endothelium rendered more permeable than normal by irradiation.

The foam cell plaques in irradiated small arteries closely resemble the early lesion of atherosclerosis (Leary [31]).

24. Flaskamp,[1d] plate XXV facing p. 82; Ellinger,[1e] 1935, fig. 9, p. 19; 1941, fig. 9, p. 24.

25. Obersteiner: Arb. a. d. neurol. Inst. a. d. Wien. Univ. **12**:96, 1905; cited by Colwell, H. A., and Russ, S.: Radium, X-Rays and the Living Cell, London, G. Bell & Sons, Ltd., 1924, p. 218.

26. Alpers, B., and Pancoast, H.: Am. J. Cancer **17**: 7, 1933.

27. Baermann, G., and Linser, P.: München. med. Wchnschr. **51**:918, 1904.

28. Montgomery, H.: Pathologic Histology of Radiodermatitis, in MacKee, G. M.: X-Rays and Radium in the Treatment of Diseases of the Skin, Philadelphia, Lea & Febiger, 1938.

29. Haendly, P.: Strahlentherapie **12**:1, 1921.

30. Windholtz, F.: Strahlentherapie **59**:662, 1937.

31. Leary, T.: Arch. Path. **32**:507, 1941 (fig. 1A).

MADUROMYCOSIS OF THE HAND

WITH SPECIAL REFERENCE TO HERETOFORE UNDESCRIBED FOREIGN BODY GRANULOMAS FORMED AROUND DISINTEGRATED CHLAMYDOSPORES

DOUGLAS SYMMERS, M.D.
General Director of Laboratories, Department of Hospitals, City of New York
AND
ANDREW SPORER, M.D.
Resident in Surgery, Goldwater Memorial Hospital
NEW YORK

Maduromycosis, or mycetoma, madurosis or, as it is perhaps most widely known, madura foot, is a fungous disease which was first adequately described by Van Dyke Carter. He encountered it as an endemic infection in and around the City of Madura in the Madras Presidency of India. Since then it has been recognized in different parts of the world, including other districts in India, and in Ceylon, Cochin China, the Netherland East Indies, Africa, Argentina, Cuba, the United States and Canada. It occurs oftenest in arid tropical or subtropical climates among men engaged in agricultural pursuits who are in the habit of working barefoot. The disease is seldom seen in women. One of the commonest modes of infection is through the pricks of thorns. Unlike actinomycosis, which is most frequently found in cattle and swine, maduromycosis is apparently confined to man.

Infection in actinomycosis is transmissible from one part of the body to another, for example, from the jaw to the intestine, while maduromycosis remains localized in the part originally affected. The disease is slowly progressive and may persist for many years. The period of incubation is not definitely established, but appears to be from two weeks to three months. In 1 instance it was estimated at twenty-five years, although this is scarcely credible. During the interval of incubation the wound of entrance may heal completely. This has no deterrent effect on the causative fungus, which in most instances is an aerobic micro-organism but which seems none the less capable of extracting from closed tissues sufficient oxygen for its purpose.

Maduromycosis most often affects the foot. Occasionally the disease involves the region of the knee, the thigh, the buttocks and, apparently most rarely of all, the hand. It appears that the case presented in this report is the first one of maduromycosis of the hand to be described in the United States and the second on the North American continent, Ocaranza having observed a case in Mexico. The infection of the hand recorded here is remarkable because it occurred in a man of 67 years who had never been outside the city of New York. The disease developed about three weeks after he fell on a wooden floor and sustained multiple abrasions of the palmar surface, through which presumably the infective fungus entered. In the literature on maduromycosis from the year 1876 to 1941 we found reports of only 4 cases involving the hand—1 case occurring in India, 1 in Italy, 1 in Bolivia and 1 in Mexico. On excluding the present case it appears that only 4 examples of maduromycosis of the hand have been recorded in the literature on medicine during a period of sixty-five years. While it is true that madura foot is far commoner than madura hand, it is hardly believable that the hand is affected so rarely as the preceding figures indicate. It is probable that many cases of maduromycosis of the hand have been observed that were never recorded.

Chalmers and Archibald [1] maintained that cases of mycetoma are divisible into two groups—those of maduromycosis and those of actinomycosis—in other words, that maduromycosis and actinomycosis are different forms of the same disease. It is shown in this paper, we believe, that maduromycosis and actinomycosis are different diseases and that the histology of maduromycosis is distinctive. According to the classification of Chalmers and Archibald, the fungi in the group

From the Third Medical Service (Dr. Thomas A. McGoldrick, director) and the Laboratories of Pathology, Goldwater Memorial Hospital, Department of Hospitals, New York.

1. Chalmers, A. J., and Archibald, R. G.: New Orleans M. & S. J. **70**:455, 1917-1918.

of maduromycosis produce granules which are "composed of large segmented mycelial filaments possessing well-defined walls" and thick-walled pigmented chlamydospores. The latter are rounded or oval and measure from 8 to 30 microns in diameter. The fungi in the subgroup of actinomycosis are provided with "very fine non-segmented mycelial filaments, in which the walls are not well-defined and in which chlamydospores are absent." The granules extruded from the affected tissues may be white or yellowish, or red or black. Each color may be produced by different types of fungi, and the same fungus may produce either the white or the black variety of granule. The red granule is rare. It is apparent that cases of mycetoma cannot be acceptably classified according to the color of the granules but only on the botanic characteristics of the fungus.

In a review of the literature to May 1941 we found reports of 38 cases of maduromycosis that had been observed in the United States. According to Jones and Alden,[2] of 26 cases reported in the United States up to the year 1931, white or yellow granules were found in 21 and black granules in 5. Eight of these cases belong apparently to the group of maduromycosis and 2 to the group of actinomycosis. The remaining 16 cases could not be satisfactorily classified because of inadequate cultural or morphologic data. Gammel[3] expressed the belief that only 6 genuine examples of maduromycosis had been identified in this country up to March 1927 and that the great majority of cases recorded under the caption of maduromycosis were in reality examples of actinomycosis. Since then Harrold and Hanan and Zurett have added 2 cases. In the case recorded here, black granules were present in the mucopurulent exudate, and chlamydospores were identified in histologic and cover slip preparations, so that the disease appears to be an example of maduromycosis. Moreover, the tissue changes in the immediate vicinity of the chlamydospores were entirely different from those produced by the ray fungus in actinomycosis. From this it appears that only 9 cases of maduromycosis have been described in the United States, including the 6 cases accepted by Gammel, the 2 reported by Harrold and Hanan and Zurett and the 1 case recorded in this report. It is probable that other cases have been observed but not recorded in the interim of seventeen years between 1927 and 1944.

2. Jones, J. W., and Alden, H. S.: J. A. M. A. **96**:256, 1931.

3. Gammel, J. A.: Arch. Dermat. & Syph. **15**:241, 1927.

REPORT OF A CASE

A. N., a white man aged 67, a clerk, was admitted to the medical service of Dr. Thomas A. McGoldrick at the Goldwater Memorial Hospital, July 7, 1937. The patient stated that in the course of the past seven years he had suffered repeated "strokes" which left him with a mild degree of weakness of the right side of the body. Two years before admission he fell and scraped his right hand on a wooden floor, "picking up many splinters." Two or three weeks later the right hand became swollen and multiple pustules appeared with black dots in them. At the time of admission the hand was greatly swollen and showed many nodular formations on both the palmar and the dorsal aspects. Some of these nodules contained sinuses which discharged yellowish pus and black granules. The skin of the hand was diffusely indurated, especially over the thenar eminences. Springing apparently from the tendon sheath of the extensor muscle of the right ring finger was a solitary cystlike formation. Throughout the rest of the hand there were multiple subcutaneous nodules, which were moderately firm in consistency, movable, painless and not tender. For weeks at a time the inflammatory changes remained in an apparently quiescent state. At other times acute exacerbations occurred without obvious cause, the nodules becoming enlarged, hot, red and tender, and the hand as a whole increasing in size. On one occasion the patient was treated with large doses of potassium iodide over an extended period without beneficial results. On the contrary, within ten days after the commencement of treatment both pustules and granules appeared to be "remarkably increased." On another occasion iodides were given for a few days and were almost immediately followed by great enlargement of the hand. The nodules increased in size to an alarming extent and were red and edematous. There was no detectable increase in the number of black granules.

Scattered over both surfaces of the right hand at the time of writing (1944) are numbers of discrete, freely movable, painless and nontender nodules varying in diameter from a few millimeters to 1 or 2 cm. Over many of them the skin is speckled by minute black deposits. Other nodules are surmounted by cuplike depressions from 1 to 5 mm. in diameter, which are encrusted by the same sort of black material. They represent the openings of fistulous tracts which formerly exuded mucopurulent material containing particles that resembled grains of gunpowder. The hand is greatly enlarged, deformed and almost useless. The affected hand measures 11.3 cm. transversely and 5 cm. in depth. The opposite hand measures 8.8 cm. transversely and 3.1 cm. in depth. Neither hand is increased in length (fig. 1).

The patient is partially paralyzed on the right side. The blood pressure varies from 220 systolic and 100 diastolic to 210 systolic and 150 diastolic. The Wassermann and Kline reactions were negative.

The duration of the disease was about seven years.

Roentgenologic Observations.—Roentgenographic examinations of the right hand were made at intervals during a period of five years between 1939 and 1944 by Dr. Henry K. Taylor, roentgenologist to Goldwater Memorial Hospital.

In 1939 roentgenograms showed areas of decalcification in the semilunar bone and os magnum and an area of ossification in the soft tissues adjacent to the metacarpophalangeal articulation of the thumb. An

osteophyte was observed at the articular margin of the distal phalanx of the thumb and productive changes at the upper and lateral margin of the proximal phalanx of the third finger. The soft tissues of the hand and those about the proximal interphalangeal articulations of the ring and middle fingers were considerably swollen.

In 1940 Dr. Taylor reported that the changes were essentially the same as those that had been observed five months before in 1939. In the course of the next three years the changes progressed noticeably. They included productive periostitis of the shafts of the second, third, fourth and fifth metacarpal bones and of the proximal phalanx of the middle finger. There was uneven density of the proximal ends of the second, third and fourth metacarpal bones with areas of rarefaction in the heads of these bones (fig. 2 *A*).

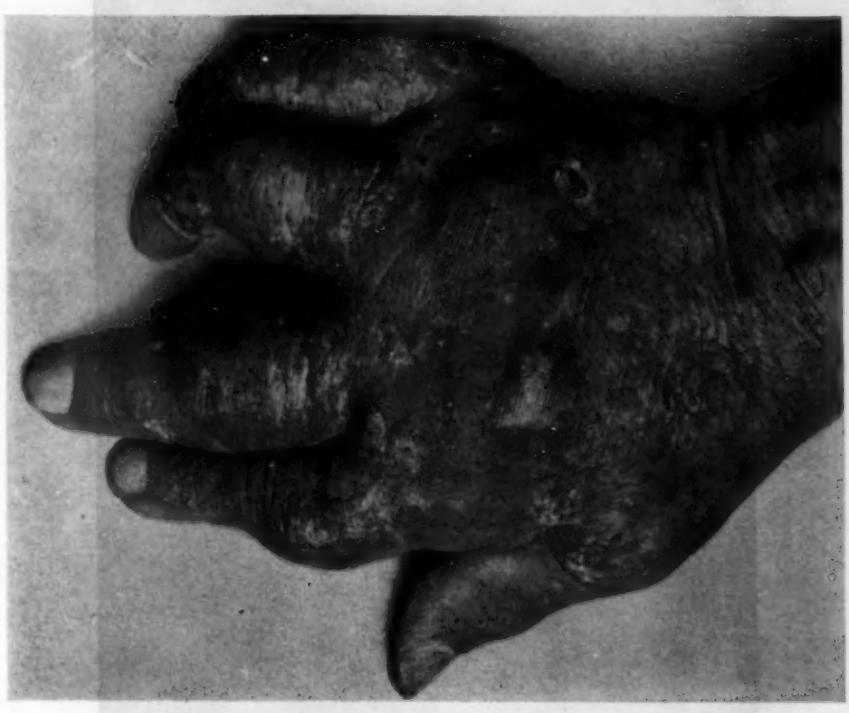


Fig. 1.—Right hand showing the black granule variety of maduromycosis, with multiple nodules and superficially healed sinuses capped by black material corresponding to deposits of pigmented chlamydospores.

In 1944 marked evidences of progression were noted. Productive periosteal changes now involved the shafts of the second, third, fourth and fifth metacarpal bones and the proximal phalanges of the middle, ring and fifth fingers. The cortex of the proximal half of the fourth metacarpal bone was eroded. Rarefaction and cystic changes were observed in the second, third and fourth metacarpal bones and in the proximal phalanx of the ring finger. The proximal portion of the middle phalanx of the fifth finger showed productive periosteal changes. The swelling of the soft tissues was far more extensive than had been noted on any previous occasion, involving the entire hand and all of the fingers with the exception of the thumb. The swellings around the fingers were fusiform, and those on the dorsum of the hand were nodular (fig. 2 *B*).

Histologic Observations.—A nodule removed for biopsy measured approximately 1 cm. in diameter. Microscopically, it was almost completely surrounded by a thick layer of collagenous fibers in the interstices of which were large numbers of "foam cells" with well defined limiting membranes, centrally placed vesicular nuclei and reticulated cytoplasm. Foam cells were also distributed in vast numbers through the underlying granulation tissue. They were arranged diffusely or in lobular formation. Some of the lobules were small and circumscribed by cellular connective tissue. Others were large, circumscribed and partially replaced by fibrous bands, which in occasional instances were thick and hyalinized. Fibrous replacement of the lobules of foam cells is probably an attempt to effect healing. In some quarters it is held that foam cells are peculiar to the tissues in actinomycosis. In our experience this is not true. On the contrary, we have encountered them in a wide variety of inflammatory lesions. In hematoxylin and eosin preparations they are indistinguishable histologically from embryonal fat cells. In this case their presence obviously could not be anticipated prior to microscopic examination, so that all of the small amount of excised tissue was used for other purposes, and none was available for frozen sections and special stains for fat (fig. 3 *A*).

The center of the excised nodule was occupied by irregularly vascularized granulation tissue, scattered through which were variable numbers of polymorphonuclear leukocytes, many eosinophils, lymphocytes and a few plasma cells, fibroblasts, isolated giant cells of the Langhans type, hordes of foam cells, Russell's fuchsin bodies and large mononuclear cells of indeterminate nature containing black pigment which did not respond to Perl's reagent for hemosiderin or to Fontana's stain for melanin. Sections impregnated with silver showed a delicate network of argyrophilic reticulum.

The most striking feature in the histologic picture is to be seen in the form of focal lesions which we have chosen to call maduromycotic "granulomas" in contradistinction to the "granulomas" of actinomycosis. For descriptive purposes the granulomas of maduromycosis may be divided into three types of different ages, all of them built around pigmented chlamydospores, which in some places are well preserved and in other places are necrotic. The age of each of the three types was estimated partly on the basis of the staining qualities of the giant cells when these were present, with special reference to the avidity with which the nuclei take up hematoxylin in sections counterstained by eosin, and partly on the degree of connective tissue encapsulation. If giant cells are absent or are present to the number of only one or two, it is assumed that the granuloma is the least mature of the three. If both cytoplasm and nuclei stain lightly and if the nuclei when present are small and well formed, it is assumed that the granuloma is of an intermediate grade of maturity. If both cytoplasm and nuclei stain well and the nuclei are large and conform more or less faithfully to the normal, the granuloma is assumed to have reached maturity. Many of the granulomas in maduromycosis show a tendency to heal spontaneously through the process of encapsulation by connective tissue. It is assumed, if indeed it is not self evident, that a greater degree of connective tissue encapsulation indicates a greater degree of maturity.

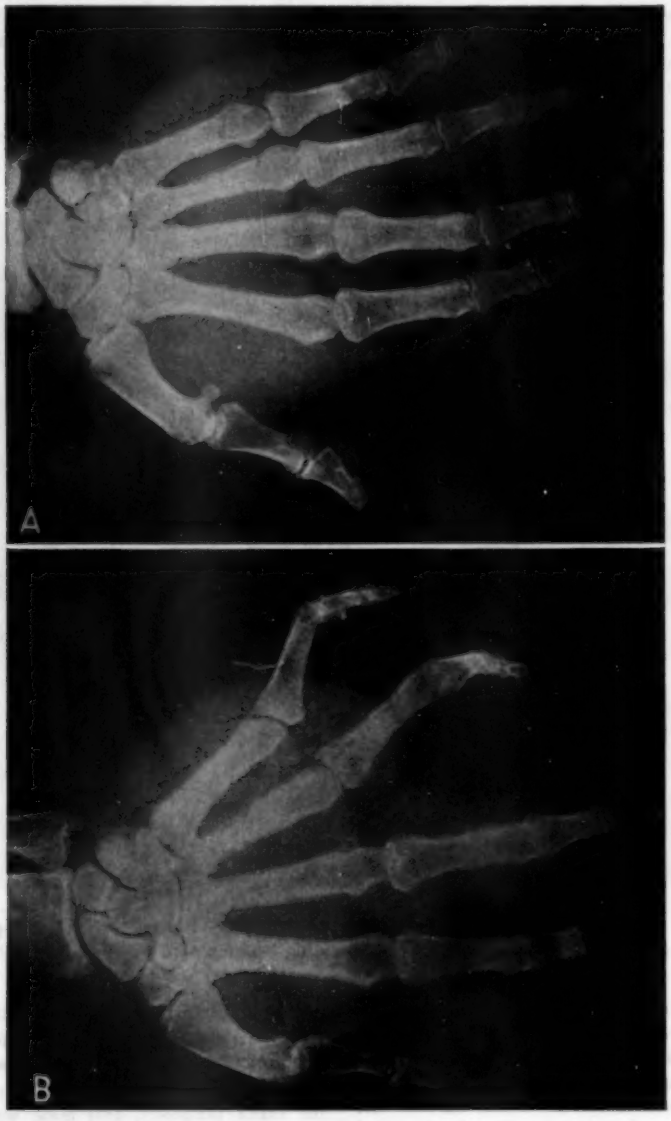


Fig. 2.—*A*, roentgenogram of the hand as it appeared in 1939, showing early bone changes and relatively slight involvement of the soft tissues. *B*, roentgenogram of the hand as it appeared in 1944, showing extensive bone changes and marked involvement of the soft tissues.

The first, simplest and probably youngest form of granuloma consists of one or more clumps of disintegrated and fused chlamydospores set in the midst of accumulations of polymorphonuclear leukocytes. Occasionally a few giant cells are found lying among the leukocytes at a distance from the remains of chlamydospores. In some instances clumps of de-

generate chlamydospores merge imperceptibly into broad bases composed of acidophilic material, projecting from the border of which are pinkish-staining needle-like formations representing, probably, mycelial remnants (figs. 3*B* and 4*A*). Some granulomas of this type show signs of beginning fibroblastic connective tissue encapsulation.

The second and apparently somewhat older but still immature form of granuloma is composed of mononuclear or multinuclear cells loosely arranged around clumps of degenerate and fused chlamydospores placed in the center or at one side. Some of the cells are rounded, others ovoid, still others pear shaped, angulated or otherwise curiously outlined, and all are relatively small. Some are provided with a single nucleus; others contain from two to five or more nuclei; all of them are poor in chromatin. The cytoplasm is smooth and stains faintly with eosin. Granulomas of this type may be fairly well circumscribed by immature connective tissue (fig. 4*B*).

The mature granulomas in maduromycosis are divisible into two types. Both are encapsulated. The first type of mature granuloma is poorly encapsulated. It is made up of two sorts of giant cells arranged more compactly than those in the second or intermediate type. One sort of giant cell is provided with richly chromatic nuclei and an abundance of smooth, distinctly acidophilic cytoplasm with or without clublike or "frayed out" prolongations. Giant cells of this sort almost always lie in close apposition to clumps of dustlike material obviously derived from disintegrated chlamydospores, particles of which may be seen in their cytoplasm. The remaining giant cell is rounded, occasionally pointed at one end, or blunt and "drawn out," the nuclei richly chromatic and the cytoplasm smooth and distinctly acidophilic but relatively small in amount. This variety of giant cell usually lies at a distance from the clumps of dustlike material derived from disintegrated chlamydospores (fig. 5). A second type of mature granuloma (fig. 6*A*) is encapsulated by fibrous or fibroblastic connective tissue or by a mixture of the two. The granuloma is composed of centrally placed giant cells, which lie among more or less well formed cells of the fibroblastic variety. No clefts or other defects come into view to indicate the former abode of chlamydospores. In this type it is safe to assume that chlamydospores are present but that they are concealed in such fashion as to escape sectioning by the microtome knife. A second granuloma of the same type (fig. 6*B*) is similarly encapsulated by connective tissue and is otherwise almost identical except for the fact that it shows degenerate chlamydospores

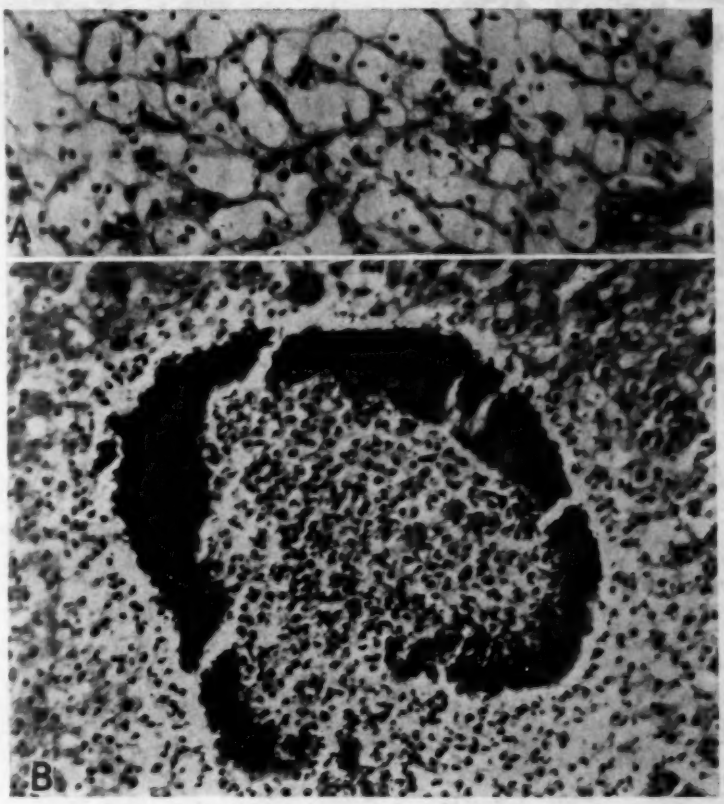


Fig. 3.—*A*, photomicrograph showing "foam cells." Hematoxylin and eosin; paraffin section; × 240. *B*, photomicrograph of a granuloma of type 1, very young, showing a C-shaped clump of densely packed or fused or degenerate and necrotic pigmented chlamydospores. The outer surface of the upper turn of the C (at right in photograph) is fringed by needle-like formations, probably degenerate mycelial filaments. The lighter-colored material on the inner side of the downward sweep of the C (at top) consists of disintegrated chlamydospores fringed on the inner side by delicate and probably degenerate mycelial filaments. Within the curve of the C and below it are collections of polymorphonuclear neutrophilic leukocytes. Note the presence of a solitary giant cell above the C. Hematoxylin and eosin; paraffin section; × 249.

in numbers gradually increasing as the giant cells containing them are sectioned at deeper levels.

There is still another type of granuloma in tissues involved by maduromycosis. It consists of spindle cells that are long, relatively broad and multinuclear. The spindle cells are arranged parallel with one another and radially around slitlike lumens which are empty or partially filled by coagulated fluid exudate. Foam cells are sometimes present in the coagulum. In other instances this type of granuloma shows multinuclear giant cells in which the cytoplasm is coarsely and irregularly granular or finely reticulated, closely resembling that of the foam cells. In one granuloma of this type a multinuclear giant foam cell was attached to the side of a cholesterol crystal, the only crystal that was found in several hundred sections, most of them cut serially. Otherwise the giant cells are indistinguishable from the Langhans variety. In granulomas of this type we have not been able to demonstrate chlamydospores. It is assumed that the clumps of

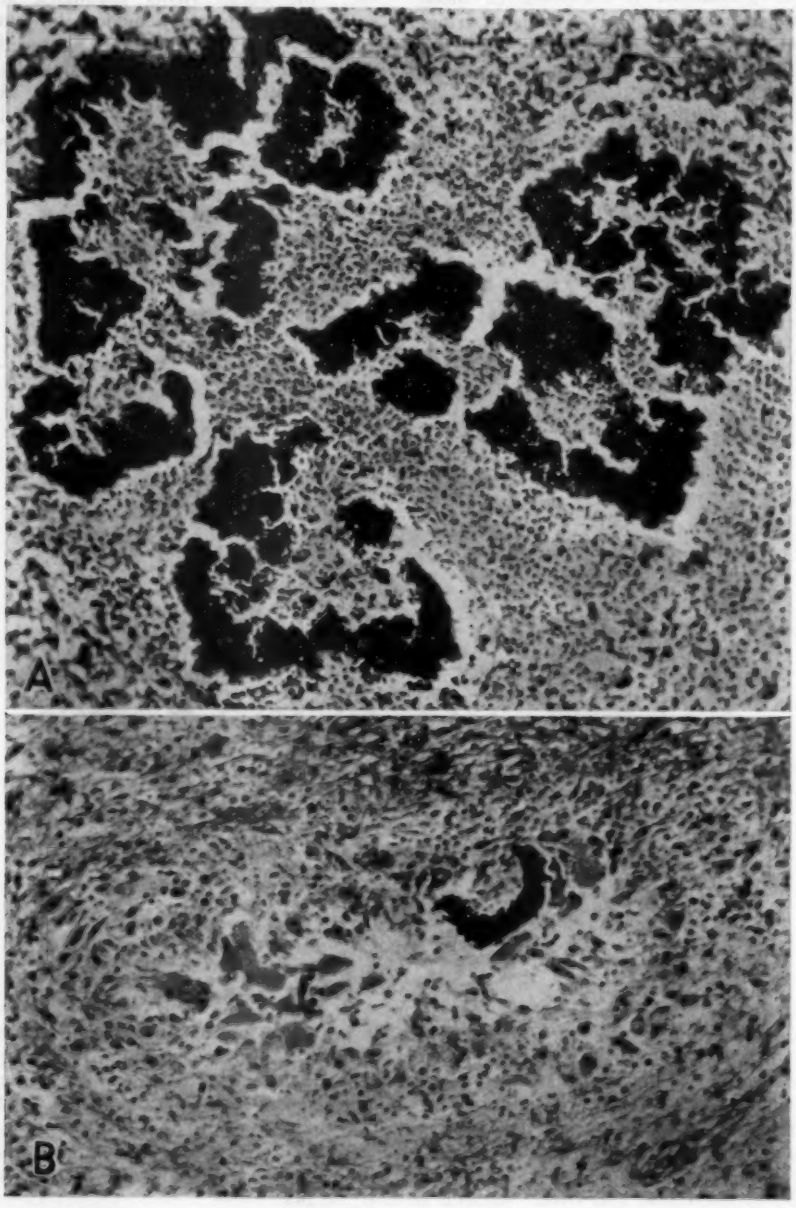


Fig. 4.—*A*, photomicrograph of a granuloma of type 1, very young, showing groups of degenerate and pigmented chlamydospores arranged in serpentine fashion and set in the midst of polymorphonuclear neutrophilic leukocytes. Note the absence of giant cells. Hematoxylin and eosin; paraffin section; × 182.

B, photomicrograph of a granuloma of type 2, the intermediate degree of maturity, showing a crescent-shaped group of degenerate and fused pigmented chlamydospores. In the immediate vicinity are numbers of curiously shaped giant cells, some of them nucleated, others anuclear, all of them staining lightly. The granuloma is circumscribed by connective tissue. Hematoxylin and eosin; paraffin section; × 200.

fused or closely packed chlamydospores are heavy and are apt to fall out in the process of embedding, or that they are so situated as to be inaccessible for sectioning. In this phase of granuloma formation the multinuclear cells are probably best interpreted as immature giant cells. Fully grown multinuclear giant cells are present in a later phase and spindle cells are numerous but seldom multinuclear. According to this conception, the granuloma is formed around hidden chlamydospores. It is possible but, we think, not probable that it might be formed around the remains of foam cells (fig. 6*C*). In this type of granuloma encapsulation occurs, but the connective tissue is often immature.

The giant cells in the granulomas of maduromycosis seem to be incapable of phagocytosing intact chlamydospores. When the latter are destroyed, the resulting granular debris is taken up by giant cells and to a lesser extent by large mononuclear cells. At intervals clumps of chlamydospores are to be seen lying free in the tissues without any especially noteworthy reaction around them. In none of the granulomas were mycelial filaments detected by examination of sections stained by the method of Gram.

Bone changes in maduromycosis seem not to have been investigated from the microscopic standpoint. It may be assumed that they do not differ materially from those in the soft tissues. The appearance to the naked eye and the roentgenologic appearance are better known and indicate various gradations of productive inflammatory lesions of the periosteum to rarefaction and caries of both the compact and the cancellous tissues. In the paper by Boyd and Crutchfield[4] there is a photographic reproduction of the bone changes in a case of maduromycosis of the foot taken from a specimen in the museum at McGill University. It is a remarkable illustration of the extent to which bone may be implicated.

Mycologic Observations.—Fresh tissues removed at operation were planted in dextrose broth and on dextrose agar slants. Dextrose agar, melted and cooled to 40 C., was inoculated to create semianaerobic conditions. All inoculated mediums were kept for thirteen weeks at 37 C. Black granules, also fresh, were planted on maltose agar, Sabouraud and corn meal medium and kept at 37 C. and another set at room temperature. Transplants were made every week for ten weeks. No growth was obtained in any of them except Staphylococcus aureus and nonhemolytic streptococcus. Six weeks later an attempt was made to cultivate the fungus by inoculating various mediums with black granules removed from an ulcerated area on the back of the patient's hand. Cultures were made as follows: in nutrient broth and on agar, both enriched with 6 per cent dextrose; in nutrient broth and on agar, both enriched with 6 per cent maltose; on liver medium enriched with 6 per cent dextrose; on Sabouraud's medium and on Bordet-Gengou agar. All mediums were subjected to both aerobic and anaerobic conditions at 37 C. and at room temperature. With the exception of the Bordet-Gengou agar, one set was kept at 37 C. and a duplicate set at room temperature. The Bordet-Gengou medium was incubated at 37 C. Subcultures

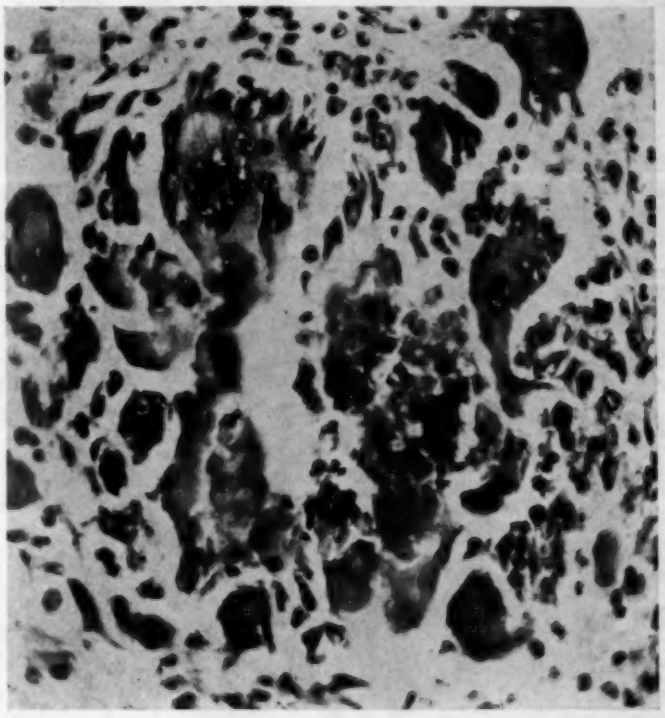


Fig. 5.—Mature granuloma showing at its center clumps of debris derived from necrotic and disintegrated chlamydospores. The cytoplasm of the giant cells in immediate juxtaposition to the clumps of debris contains phagocytosed material. Note the mature, comparatively well formed, well nucleated giant cells at the periphery. Hematoxylin and eosin; paraffin section; × 414.

4. Boyd, M. F., and Crutchfield, E. D.: Am. J. Trop. Med. **1**:215, 1921.

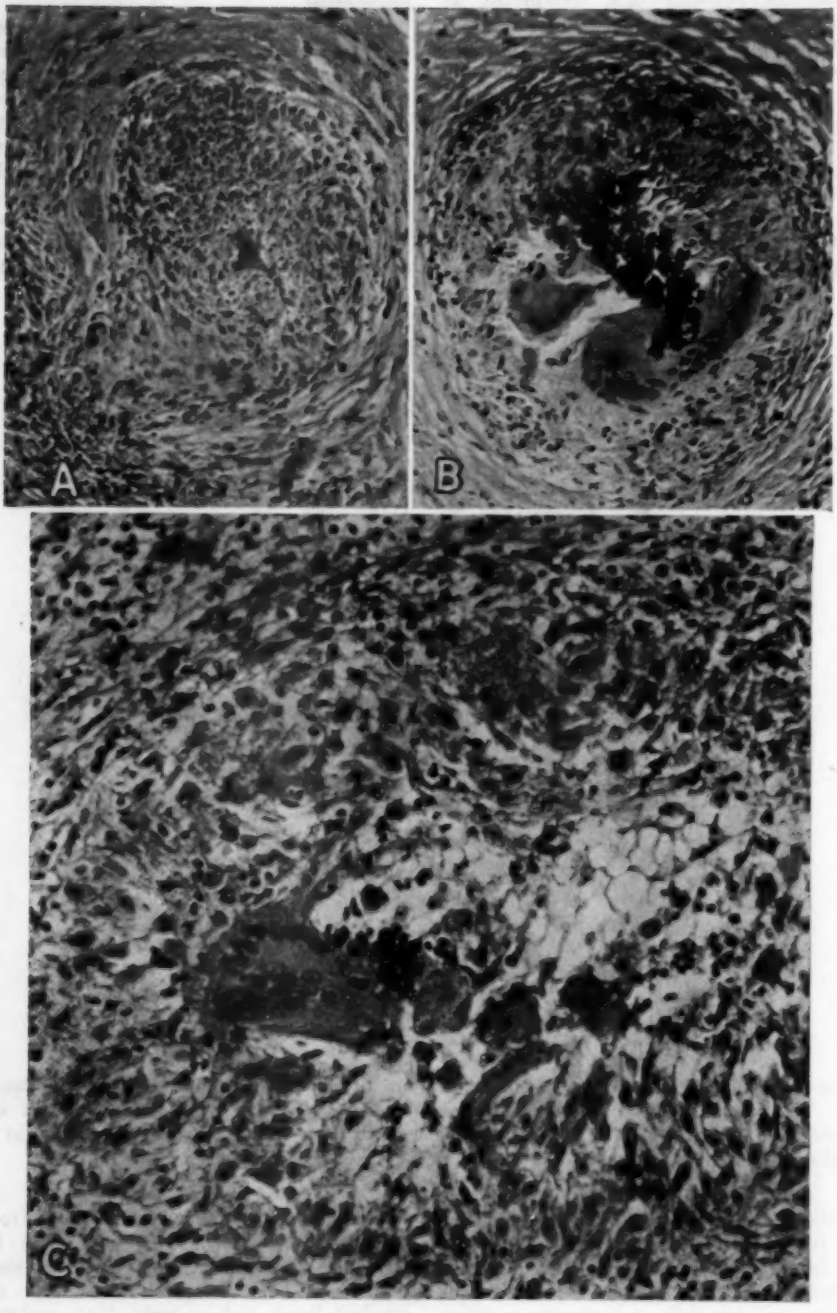


Fig. 6.—*A*, photomicrograph showing an encapsulated mature granuloma. Note the absence of chlamydospores. Hematoxylin and eosin; paraffin section; × 150. *B*, photomicrograph showing an encapsulated mature granuloma formed around disintegrated chlamydospores lying partly in the cytoplasm of a giant cell. Hematoxylin and eosin; paraffin section; × 150. *C*, photomicrograph of a granuloma partially encapsulated by cellular immature connective tissue. Note the absence of chlamydospores. Near the center and to the right the reticulated structures represent the limiting membranes of foam cells. Hematoxylin and eosin; paraffin section; × 288.

were made every seven days. Except for nonhemolytic streptococcus and Staph. aureus no growth was obtained at the end of five weeks. Four weeks later still another attempt was made, the same methods being used, but without success. On three different occasions minute particles of black grains freshly removed from the tissues of the patient were flooded with 10 per cent sodium hydroxide solution and crushed beneath cover glasses. Microscopic examination showed tangled masses of sharply defined segmented mycelial filaments and innumerable thick-walled pigmented chlamydospores. Fungi belonging to the group of maduromycosis are difficult, sometimes impossible to cultivate. This is said to be especially true of the black grain variety (La Dantec). Nevertheless it is our purpose to try for another six months or a year before the attempt is abandoned.

COMMENT

Although a great deal of mycologic work has been done on the various species of fungi concerned in the production of maduromycosis, comparatively little attention has been given to the anatomic and histologic changes. In addition to destruction of skin, fat, tendons, muscle tissues and bone, anatomic changes are present in the form of subcutaneous nodules, fistulous tracts and sinuses discharging mucopurulent material in which there are white, black or red granules, depending on the type of fungus.

Over seventy years have elapsed since maduromycosis was described. According to Boyd and Crutchfield,[4] up to the year 1921 not more than a half dozen contributions had been made to the histology of the disease. Since then a few observations have been added, including those of Boyd and Crutchfield, Jones and Alden [2] and Tribedi and Mukherjee.[5] We have inquired into this aspect with special care because it seemed inconceivable that such a spectacular array of histologic changes could have been overlooked for such an extended length of time. This apparently inexcusable oversight is reconcilable with the fact that maduromycosis has been investigated almost exclusively by those who were primarily concerned with its mycology. None of the observations with which we are familiar includes a description of chlamydospores in the tissues involved in maduromycosis and the formation of granulomas around them.

Because of military activities in India and in other localities where the disease is prevalent it is to be expected that numbers of men will be invalided home with maduromycosis. Physicians will undoubtedly be on the alert for its appearance in this and other countries where it has been heretofore almost unknown. It seems that the sulfonamide drugs have not been used in the treatment of maduromycosis. If the disease is recognized sufficiently early curettage or excision of individual nodules as they arise should be done in an effort to remove all of the diseased tissue, followed, perhaps, by local application of one of the sulfonamide compounds. In the later stages, more especially if the disease has penetrated bone, amputation is advocated by those who are most experienced in its treatment.

5. Tribedi, B. P., and Mukherjee, B. N.: Brit. J. Surg. **27**:256, 1939.

SUMMARY AND CONCLUSIONS

The case presented in this report appears to be the first example of maduromycosis of the hand to be described in the United States, the second on the North American continent and the fifth thus far recorded. It is probable that additional cases have been observed in this and other parts of the world but not recorded.

It is believed that foreign body granulomas formed around chlamydospores in the tissues involved in maduromycosis have not been previously described. Of these there are three types. Most of them are encapsulated. One type is immature and the disintegrated chlamydospores in it are displayed against a background composed almost entirely of polymorphonuclear neutrophilic leukocytes. Encapsulation, if present, is ill defined. The second or intermediary form contains clumps of disintegrated chlamydospores and young giant cells, many of which present curious configurations and are poor in nuclear chromatin. Granulomas of this type are often encapsulated, usually by young connective tissue. The third type of granuloma is mature. The giant cells in it engage in phagocytosing waste material derived from the disintegration of chlamydospores. In some instances the mature granuloma is encapsulated by well organized connective tissue; in others, by connective tissue that is cellular and obviously young.

There is a type of granuloma in which, we assume, either chlamydospores were originally present but dropped out in the process of embedding or the chlamydospores around which it is formed lie beyond the path of the microtome knife. It is possible but, we think, not probable that this type may be formed around the remains of foam cells. It consists of broad multinuclear spindle cells arranged around slitlike lumens or of a combination of giant and spindle cells arranged around the collapsed membranes of foam cells. Granulomas of this type are sometimes encap-

sulated by young connective tissue. Finally, there are numerous instances in which chlamydospores lie free in the tissues involved in maduromycosis without any noteworthy reaction around them.

Encapsulation of the granulomas in maduromycosis is obviously indicative of an attempt to promote healing. Fibrous replacement of foam cells may be interpreted as an effort in the same direction. This statement applies also to the argyrophilic reticulum which is liberally distributed through the nodules. Precedents for the latter conclusion are set by those young epithelioid tubercles which are partly or almost completely replaced by overgrowth of argyrophilic reticulum [6] and by the nodules of Kaposi's fibroblastic sarcoma [7] showing overgrowth of the same sort of reticulum.

Histologically maduromycosis could scarcely be confused with any other known disease, more especially with actinomycosis. The granuloma of maduromycosis is formed around degenerate chlamydospores; that of actinomycosis, around ray fungi. Both are foreign body reactions and bear only a remote resemblance to one another. The causative fungi are closely related, but the diseases are different from the clinical, mycologic and histologic aspects. It seems to us that the classification of Chalmers and Archibald should be abandoned on the ground that it commits one to the belief that maduromycosis and actinomycosis are different forms of the same disease.

6. Symmers, D.: Arch. Path. **31**:304, 1941.

7. Symmers, D.: Arch. Path. **32**:764, 1941.

STUDIES IN VITRO ON THE PHYSIOLOGY OF NORMAL AND OF CANCEROUS CELLS

II. THE SURVIVAL AND THE GLYCOLYSIS OF CELLS UNDER AEROBIC AND UNDER ANAEROBIC CONDITIONS

MAJOR ROBERT SCHREK

MEDICAL CORPS, ARMY OF THE UNITED STATES

One of the earliest methods of studying the physiology of animals was the determination of the factors necessary for their survival. It was known, for example, that the whale is not a fish since it is unable to live submerged in water for any considerable period. Similarly, animals were known to differ in regard to the type of food necessary for their survival and could be thus classified as carnivorous, herbivorous and omnivorous. Evidently the old method of observing the ability of animals to survive in normal and in artificial environments was useful in the classification of animals and in the demonstration of fundamental differences in the physiology of different species. Possibly the most important function of the method was the delineation of those factors which were essential for survival and which therefore required further investigation.

By analogy a determination of the capacity of cells to survive in various artificial environments should be useful in a study of cellular physiology. The survival of cells in vitro can be quantitatively studied by the method of unstained cell counts.[1] The present work consists of a preliminary survey to determine whether the survival of cells in vitro is dependent on such factors as (1) the type of cell, (2) the species of animal used and (3) the presence or the absence of air or of dextrose or of both. A few observations were also made to determine whether the survival of cancerous cells depends primarily on the type of the ancestral cell or on its malignancy.

The experiments were controlled by routine determinations of the hydrogen ion concentrations of the suspensions. It soon became apparent that the incidental observations on the hydrogen ion concentrations were as interesting as the principal findings on the survival of cells. It seemed that the changes in p_H were a quantitative measure of the utilization of dextrose by the cells. The present findings can be correlated with those obtained by chemical and manometric methods on aerobic and anaerobic glycolysis.

From the Tumor Research Unit, Veterans Administration.

The paper is published with the permission of the Medical Director of the Veterans Administration, who assumes no responsibility for the opinions expressed or the conclusions drawn by the author.

1. Schrek, R.: Am. J. Cancer **28**:389, 1936.

METHODS

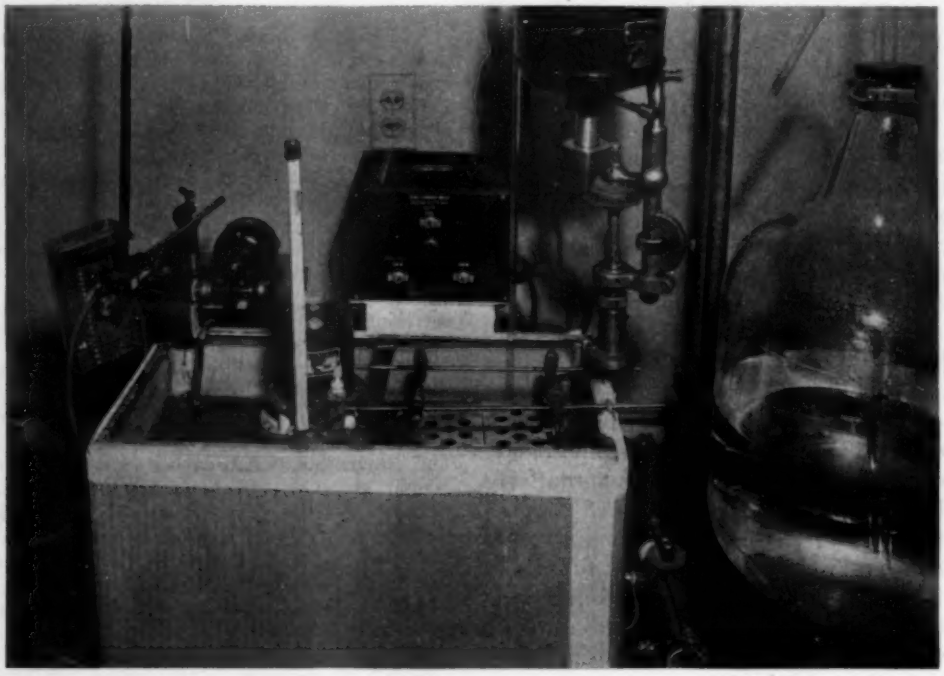
Viable cells were obtained from many sources. Thymus and testicle were minced with sharp scissors and forced through 80 mesh Monel metal wire gauze. Leukocytes were obtained by injecting aleuronat (an albuminoid substance and lecithin) intraperitoneally in 5 to 10 per cent suspension and collecting the peritoneal fluid in sixteen hours. Blood was obtained from three types of patients: those with leukemia, those with hypertension and those with polycythemia. After the addition of sodium citrate or heparin, the blood was centrifuged, and the buffy coat was withdrawn. Human tissue was obtained directly from the operating room or at autopsies performed one hour after death.

The suspensions of cells obtained by these methods were washed three or more times with a phosphate–Ringer solution, which usually contained 20 per cent of a fifteenth-molar phosphate buffer with a p_H of 7.7. The Ringer solution used had a low concentration of calcium chloride (0.01 per cent) to permit addition of the large amount of buffer without precipitation of calcium salts. A small amount of the solution described was added to the washed cells. Half of the suspension was then diluted with an equal volume of the same solution and the other half with this solution plus 0.2 per cent of dextrose.

For aerobic incubation, test tubes (100 by 13 mm.) with 0.2 cc. amounts of suspension were maintained in a water bath at 45 C. and were shaken horizontally one hundred and fifty times a minute (figure). The numbers of stained, unstained and red blood cells in the suspension were counted by means of a hemocytometer after the addition of eosin (3.8 cc. of a 1:1,000 solution in Tyrode's fluid was added to 0.2 cc. of suspension). In some cases a Petroff-Hauser counting chamber was used and the preparation examined with an oil immersion lens. The results were expressed in cells per millimicroliter (0.000000001 liter, or 0.001 cu. mm.). This unit of volume was adopted instead of the commonly used cubic millimeter to avoid the use of unnecessarily large numbers. A blood count of 5,000,000 erythrocytes and 6,500 leukocytes per cubic millimeter would be expressed by the present method as 5,000 and 6.5 cells per millimicroliter.

The findings on the survival of the cells were represented on semilogarithmic paper. The graphs simplified a comparison of the results and enabled one to estimate the 10 per cent survival period, i. e., the time required to kill 90 per cent of the viable cells.

To incubate the suspensions under anaerobic conditions, glass tubing, 4 mm. in outside diameter, was cut into lengths of 14 cm., and short pieces of rubber tubing were attached to one end. The glass tubing was then filled completely with suspension and sealed by slipping the rubber tubing over the other end of the glass tubing. The preparation was inspected to be sure that no air had been entrapped. It was then placed in a metal box (6 by 3 by 1 inches [15 by 7.5 by 2.5 cm.]), which was rotated end over end in the water bath at 30 revolutions per minute. The apparatus used is shown in the accompanying figure. After incubation, the rubber tubing was removed from both ends and the suspension transferred to a test tube. This technic was used to avoid contaminating the test fluid with any excess suspension that may have been sucked into the rubber tubing. A count was made on the anaerobic suspension as described for the aerobic preparation.



Water bath and accessories used for the incubation of suspensions of cells under aerobic and under anaerobic conditions.

Test tubes with the aerobic suspensions are shaken horizontally in the rack by means of a bar which is attached to an eccentrically placed screw on a disk. The large motor on the right causes the disk to revolve at a slow rate of speed. The rack is suspended from two parallel bars by four pulleys.

The perforated metal box is used to carry the glass tubing with the anaerobic suspensions. The box is lowered into the water bath and rotated at 30 revolutions per minute by means of a belt made of rubber tubing. The belt is attached to the pulley of the motor on the left. On top of the electric relay has been placed an anaerobic preparation.

The p_H of each suspension before and after incubation was determined by means of an electrometer equipped with a microchamber and a glass electrode. With this apparatus it was possible to obtain the p_H of 0.4 cc. of suspension.

No formal experiment was attempted to determine the experimental error of the methods used. It was observed, however, that there was no major discrepancy in the findings from day to day, although minor variations, of course, occurred. Ten per cent survival times were considered significantly different if one was at least 50 per cent larger than the other. A difference in p_H of 0.20 in the same experiment or 0.40 in different experiments was accepted as significant.

OBSERVATIONS

Survival of Different Types of Cells of the Rabbit.—Cells derived from the rabbit were suspended in a dextrose–phosphate–Ringer solution and maintained at 45 C. under aerobic conditions. The findings on the survival of cells derived from the thymus, the testicle, a hyperplastic cervical lymph node and a peritoneal exudate are presented in table 1. The table shows that the cells of the lymph node had the shortest 10 per cent survival period (one and one-tenth hours), the thymic cells the longest (three and four-tenths hours) and the testicular cells and the leukocytes of the peritoneal exudate an intermediate period (one and eight-tenths and two and eight-tenths hours, respectively). It is to be noted that the cells derived from the lymph node and those from the thymus were predominantly lymphocytes. In spite of the similarity in morphologic appearance, the cells from these two tissues showed marked differences in survival in vitro. Some incomplete experiments on suspensions from the spleen suggested that the large and the small cells of this organ differed in period of survival. It appeared that the large lymphocytes remained viable a shorter period than the small ones.

It is to be concluded from the data presented in table 1 that cells from different tissues of the rabbit

varied markedly in their capacity to survive in vitro under identical conditions.

Survival of Cells from Different Species.—The effect of species on the capacity of thymic cells to survive was studied by using tissues from the mouse, the rat, the rabbit and the sheep. The results are summarized in table 1. It is seen from the table that thymic lymphocytes from four species of animals had approximately the same capacity for survival in vitro. The differences in the 10 per cent survival periods are small and cannot be considered definitely significant. The findings permit the conclusion that there are probably no large differences in the survival periods of the thymic cells of the mouse, the rat, the rabbit and the sheep.

The 10 per cent survival periods of testicular cells of the rat, the rabbit and the sheep were one, one and eight-tenths and one and three-tenths hours, respectively. The variation is not definitely significant.

The survival curves of leukocytes derived from peritoneal exudates of the rat and the rabbit are shown in the table. It is seen that the 10 per cent survival periods of the cells of the rat and the rabbit were approximately the same (two and five-tenths and two and eight-tenths hours, respectively). The leukocytes from normal human blood and from a cervical abscess of man survived in vitro for many hours. It is realized that the leukocytes from these two sources are not necessarily homologous to the cells of the aseptic peritoneal exudate.

In table 1 it is seen that cells from a hyperplastic lymph node of a rabbit had a short survival period (one and one-tenth hours) whereas those from a human lymph node were capable of surviving a long time (four and nine-tenths hours). Cells of nodes from 3 other patients also had prolonged survival periods. The marked variation in the survival periods of cells from rabbit and human lymph nodes may be due to the difference in species, or it may be the result of a difference in the inflammatory or hyperplastic processes of the nodes studied.

The present experiments do not provide any definite answer as to the effect of species on the survival periods of homologous cells. Evidently a wider variety of tissues and species should be studied. It is to be noted, furthermore, that the present experiments were designed to detect only major variations in survival periods. The present findings suggest that the type of cell is more important than the species of animal in determining the survival period of the cell.

TABLE 1.—*Survival of Normal, Inflammatory and Neoplastic Cells in Vitro at 45 C. in the Presence of Oxygen and Dextrose*

Source of Cells (Tissue and Species)	Cells per Millimicroliter in Original Suspension			Percentage of Unstained Cells Remaining Viable at Given Hour of Incubation							Estimated 10% Survival Time, Hr.
	Unstained Cells	Stained Cells	Red Blood Cells	1 Hr.	2 Hr.	3 Hr.	4 Hr.	5 Hr.	6½ Hr.	8 Hr.	
Thymus											
Rabbit	92	35	4	85	42	21	4.0	..	..	..	3.4
Rat	118	9	3	61	28	7.3	0.4	..	..	..	2.8
Mouse	51	13	4	102	66	33	..	..	..	..	...
Sheep	57	63	2	23	..	13	6.8	2.0	..	..	4.1
Lymph node											
Rabbit	73	16	3	17	0.1	..	..	..	..	..	1.1
Man	89	30	6	109	..	91	..	..	1.2	..	4.9
Testicle											
Rabbit	57	14	10	76	3.9	..	..	..	..	..	1.8
Rat	60	14	17	7	0.2	..	..	..	..	..	1.0
Sheep	66	32	0	38	0.5	..	..	..	..	..	1.3
Peritoneal fluid											
Rabbit	36	3	39	68	53	7.0	1.1	..	..	..	2.8
Rat	21	2	11	..	15	6.8	..	..	..	..	2.5
Pus											
Man	20	130	26	100	..	85	31	19	..	..	...
Blood											
Man	147	4	148	100	100	50	..	..	35	..	...
Lymphatic leukemia, blood											
Man	102	9	342	67	49	..	48	..	..	10	8.0
Man	154	40	36	96	94	..	47	28	..	..	...
Man	70	31	15	103	59	80	..	62	..	..	...
Myelogenous leukemia, blood											
Man	130	1	263	64	..	46	..	30	3	..	5.4
Man	132	2	344	106	95	27	..	22	..	..	...
Eosinophilic leukemia, blood											
Man	50	1	802	90	..	60	..	34	..	..	...
Giant follicular lymphoblastoma, node											
Man	95	22	3	81	73	59	24	..	..	..	...
Hodgkin's disease, node											
Man	80	24	8	100	55	34	14	..	..	..	...
Lymphatic leukemia, subcutaneous nodule											
Mouse	189	39	6	15	0	..	..	..	..	..	1.5
R39 sarcoma											
Rat	24	56	27	42	15	2.3	..	..	..	..	2.2

Effect of Lack of Dextrose on Survival of Cells.—It has been shown that cells remained viable in the presence of dextrose and oxygen for considerable periods. The question arises whether the sugar in the medium was essential for survival. Accordingly, the 10 per cent survival times of cells in mediums with and without dextrose were compared. The difference is represented in table 2 as a percentage of the survival period of cells in the presence of oxygen and dextrose. It is readily seen that for all the normal types of cells studied the differences were small and not significant. The findings indicate that at 45 C. under aerobic conditions the cells survived as long in the absence of dextrose as in its presence. Apparently, dextrose was not essential for the aerobic survival of cells.

Effect of Lack of Oxygen on Survival of Cells.—To determine whether oxygen is essential for the maintenance of cellular viability, the 10 per cent survival periods of cells in a dextrose-containing medium under anaerobic and aerobic conditions were determined and are presented in table 2. The differences between the survival times are given in the table. It is seen that in most cases there was no appreciable difference in the aerobic and anerobic survival times of cells. In two experiments it was observed that testicular cells of the rat and leukocytes of the peritoneal exudates of the rabbit had significantly shorter survival periods when exposed to air. Other experiments on the same types of cell failed to confirm these findings. It is probable that some factor was not completely controlled. Under certain conditions exposure to air was apparently toxic to the cells.

and four-tenths hours). The cells were able to survive satisfactorily when deprived of either oxygen or dextrose but not when deprived of both reagents. No explanation can be offered for the marked difference in the behavior of the thymic cells of the rabbit as compared with those of the rat and the sheep.

In one experiment a suspension of cells from the thymus of a 2 day old rabbit was prepared. In the absence of dextrose 90 per cent of the cells became stainable in eight-tenths hour on anaerobic incubation and in two and nine-tenths hours under aerobic conditions. It is seen that the thymic cells of a very young rabbit were as sensitive to the absence of oxygen and dextrose as those of the adult animal.

It may be concluded that all the cells studied except the thymic cells of the rabbit were able to survive satisfactorily in the absence of both oxygen and dextrose.

TABLE 2.—*Effect of Lack of Dextrose, Oxygen or Both on the Survival of Cells Incubated at 45 C.*

Source of Cells (Tissue and Species)	10% Survival Time in Hours				Effect of Lack of		
	With Dextrose		Without Dextrose		Dextrose	Oxygen	Dextrose and Oxygen
	Aerobic (a)	Anaerobic (b)	Aerobic (c)	Anaerobic (d)	$100\left[\frac{c-a}{a}\right]$*	$100\left[\frac{b-a}{a}\right]$*	$100\left[\frac{d-a}{a}\right]$*
Thymus							
Rabbit	3.4	3.4	3.5	0.5	3+	0	85—†
Rat	2.8	3.0	3.2	3.1	14+	7+	11+
Sheep	4.1	4.6	4.3	4.4	5+	12+	7+
Lymph node							
Rabbit	1.1	1.0	0.9	0.5	18—	9+	55—†
Testicle							
Rabbit	1.8	1.7	1.6	1.6	11—	6—	11—
Rat	1.0	1.5	0.9	1.4	10—	50+†	40+
Rat	1.1	1.4	1.3	1.2	18+	27+	9+
Sheep	1.3	1.4	1.2	1.4	8—	8+	8+
Peritoneal exudate							
Rabbit	2.8	2.7	3.5	2.3	25+	4—	18—
Rabbit	1.3	3.2	1.4	3.3	8+	146+†	154+†
Rat	2.5	2.7	3.2	2.9	28+	8+	16+
Lymphatic leukemia, blood							
Man	8.0	(8.0)§	(8.0)§	(8.0)§	None‡	None‡	None‡
Myelogenous leukemia, blood							
Man	5.4	(7.0)§	5.8	(5.0)§	7+	Increased†	Increased†
Man	(5.5)§	(5.5)§	(5.5)§	(5.5)§	None‡	None‡	None‡
Eosinophilic leukemia, blood							
Man	(5.0)§	(5.0)§	4.5	4.4	Decreased†	None‡	Decreased†
Lymphatic leukemia, nodule							
Mouse	1.5	2.6	1.3	2.4	13—	73+†	60+†
R39 sarcoma							
Rat	2.2	2.5	2.7	2.3	23+	14+	5+

* The formulas show that the effect was measured by the difference in survival periods, expressed as a percentage of the survival period in the presence of dextrose and oxygen.

† The differences are greater than 50 per cent and are considered significant.

§ More than 10 per cent of the cells were still viable at the termination of the experiment. The numbers in parentheses show the number of hours the cells were incubated.

‡ The differences are not significant according to graphs of survival curves.

It is concluded from the present studies that oxygen was not required for the in vitro survival of cells in the presence of dextrose and that atmospheric oxygen may be toxic.

Effect of Lack of Both Oxygen and Dextrose on Survival of Cells.—It has been shown that cells were able to survive in the absence of either oxygen or dextrose. Can cells withstand the lack of both reagents? Table 2 shows the 10 per cent survival periods in the absence of the two reagents. It is seen that the cells derived from the testicle and the peritoneal exudate were capable of surviving satisfactorily in a dextrose-free medium under anaerobic conditions. Similarly the survival periods of the thymic cells of the rat and the sheep were not affected by the absence of the two reagents. In sharp contrast, the thymic cells of the rabbit survived a much shorter period of time (five-tenths hour) in the absence of both oxygen and dextrose than when these substances were present (three

Effect of Other Sugars on Anaerobic Survival of the Thymic Cells of the Rabbit.—A study was made to determine whether sugars other than dextrose can prevent the early death of the thymic cells of the rabbit under anaerobic conditions (table 3). In this study a lesser amount of buffer was used (5 per cent phosphate buffer in Ringer's solution), and the changes in the p_H were marked. After incubation for one-half hour the suspension had 123 viable cells per millimicroliter when dextrose was present and only 24 cells in its absence. Mannose was found equally effective in permitting the survival of cells (157 viable cells after incubation for one-half hour). Galactose did to some extent prolong the life of the lymphocytes (77 unstained cells). The other sugars, fructose, xylose, sucrose and maltose, did not increase the period of anaerobic survival (6 to 25 unstained cells).

It will be shown later from a study of the p_H of the suspensions that the cells can ferment dextrose and

mannose and possibly galactose. These findings indicate that the presence of a fermentable sugar prolonged the survival of the thymic cells of the rabbit under anaerobic conditions.

Microscopic Observations on Polymorphonuclear Leukocytes of Blood and Peritoneal Exudate.—The leukocytes derived from normal blood were found to differ in one important respect from the cells derived from peritoneal fluid. During the microscopic examination of the suspension before incubation, the polymorphonuclear leukocytes of the blood appeared to enlarge considerably and became translucent or even transparent. At first it seemed that the cells were undergoing swelling and lysis possibly as a result of the toxic action of the light of the microscopic lamp. Further study in a Petroff-Hausser chamber showed, however, that the polymorphonuclear leukocytes, but not the lymphocytes, had a notable tendency to flatten themselves against the glass slide of the counting chamber. In a few minutes the small unstained spherical leukocytes became large, thin and almost transparent. This tendency of the cells made it difficult to obtain an accurate count of the unstained leukocytes in the original suspension. In contrast, the stained cells in the blood and the unstained leukocytes of the peritoneal fluid maintained their shape. After incubation of the suspension at 45 C. the unstained cells of the blood lost their capacity to flatten themselves against the glass.

Survival of Neoplastic and Leukemic Cells.—Experiments were performed on the leukocytes of the blood of a few patients with lymphatic, myelogenous and eosinophilic leukemia. In 3 cases of lymphatic leukemia the cells had very long survival periods (table 1). The 10 per cent survival period in the presence of oxygen and dextrose was found to be eight hours in 1 case. The leukocytes of myelogenous and eosinophilic leukemic blood were found to have similar prolonged survival periods. It should be noted that the cells of normal human blood were also found to survive for a considerable period. No appreciable differences could be detected in the survival periods of normal and leukemic leukocytes. In 1 case of myelogenous leukemia oxygen appeared to have a toxic action on the cells (table 2).

The diseased lymph nodes in which the changes had been diagnosed as giant follicular lymphoblastoma and Hodgkin's disease were obtained from the operating room. The survival period of the cells in suspensions from these tissues had the same order of magnitude as the cells derived from hyperplastic lymph nodes (table 1).

Suspensions were prepared from a transplantable strain of lymphatic leukemia cells, Rff 27, which was grown as subcutaneous nodules in dba mice. Rff 27 was provided by Dr. Jacob Furth and Miss Mary C. Boon. Microscopic examination of the original suspensions showed small and moderate-sized cells, few of which were stainable. The 10 per cent survival time of the cells in the absence of air was two and six-tenths hours, which is somewhat less than the survival period of mouse thymus (table 2). Cells of this tumor incubated aerobically had a much shorter survival period (one and five-tenths hours with dextrose and one and three-tenths hours without dextrose) than those maintained anaerobically (two and six-tenths and two and four-tenths hours, respectively). It seemed that exposure to air was deleterious to the cells.

Suspensions derived from sarcoma R39 of the rat were found to contain large fibroblasts, which were short, thick and spindle shaped, and also round cells, which were small, medium sized or very large. The R39 sarcoma was provided by Dr. William H. Woglom. Many cells in the original suspensions were stainable with eosin. The finding of numerous nonviable cells is not surprising since the tumor on gross and histologic examination has a large necrotic center. The 10 per cent survival period of the cells of this tumor was two and two-tenths hours (table 1). The rate of

TABLE 3.—*Effect of Various Sugars on the Anaerobic Survival of Thymic Cells of the Rabbit and on the p_H of the Suspensions Incubated Anaerobically and Aerobically at 45 C.*

Sugar	Concentration of Sugar, per Cent	Unstained Cells per Millimoeroliter			p_H of Suspensions			
		Before Incubation	After Anaerobic Incubation		Before Incubation	After Anaerobic Incubation		After Aerobic Incubation,
			0.5 Hr.	1.0 Hr.		0.5 Hr.	1.0 Hr.	2.5 Hr.
None	0	168	24	10.6	7.03	7.20	6.98	7.20
Dextrose	0.1	...	123	35	6.98	6.13	5.68	5.92
Mannose	0.1	...	157	35	7.03	6.13	5.59	5.92
Galactose	0.1	...	77	7.3	6.96	6.84	6.84	7.05
Fructose	0.1	...	12.6	7.8	6.85	6.83	6.78	
Xylose	0.1	...	15.8	5.0	6.98	6.95	6.95	
Sucrose	0.2	...	6.2	1.6	6.92	6.85	6.88	
Maltose	0.2	...	25	14.0	6.98	6.92	6.88	

death was not appreciably increased or diminished by the absence of oxygen, dextrose or both.

The present experiments were not broad enough to answer definitely the question whether the in vitro survival period of cancerous cells depends on the type of ancestral tissue or on the malignancy of the cells. The findings suggest, however, that the survival period of cancerous cells in vitro has the same order of magnitude as that of homologous normal cells.

p_H of Suspensions of Normal Cells Under Anaerobic Conditions in the Presence of Dextrose.—The observations on the p_H of suspensions were made incidental to the work described in the foregoing sections. It is to be noted that the experiments were not designed to study changes in the hydrogen ion concentration. In fact, sufficient buffer was added to prevent excessive changes. The findings are of sufficient interest, however, to warrant reporting at this time.

Suspensions of rabbit thymic cells were prepared containing 0.1 per cent dextrose and having varying concentrations of thymic cells of the rabbit. The p_H values of the original suspensions were 7.2 to 7.4. After anaerobic incubation for one hour at 45 C. the p_H decreased slightly or considerably (table 4). It can be seen from the table that the greater the number of viable cells in the original suspension, the greater was the amount of decrease (92 viable thymic cells per millimicroliter decreased the p_H to 6.90; with 240 cells the p_H dropped to 6.38). After three hours of anaerobic incubation the p_H of the suspensions was lower than

after one hour of incubation. The greater the concentration of cells and the longer the period of anaerobic incubation, the greater was the amount of acid produced in suspensions containing dextrose.

In comparing the production of acid by cells, it is important to use suspensions having the same concentration of viable cells. As the observations on p_H at first were only incidental, no attempt was made to control rigidly the concentration of viable cells in the suspensions. To circumvent this difficulty, it was decided to compare the acid produced by a suspension with that developed by an equally concentrated suspension of thymic cells. First a graph was constructed showing the relationship between the number of thymic cells of the rabbit and the p_H after one hour's incubation at 45 C. It was then possible to obtain the differences between the p_H values for incubated suspensions of the cells under investigation and of thymic cells. The calculated differences in p_H are shown in table 4.

Thymic cells of the rat and the sheep caused a slightly greater decrease in the p_H of anaerobic suspensions than that produced by the same number of cells of the rabbit (table 4). The differences were, however, small and cannot be considered significant. It seemed quite definite that there was no great variation in the capacity of the thymic cells of the three species to produce acid in dextrose-containing suspensions under anaerobic incubation.

Cells of the immature testicle of a lamb caused approximately as much of a decrease in the p_H in three hours of incubation as a comparable number of thymic lymphocytes (table 4). It is to be noted that the rate of death of testicular cells was much greater than that of the thymic cells, and therefore the rates of production of acid in the two suspensions are not strictly comparable. If one makes allowance for the difference in the rate of death it would seem that the testicular cells produced a greater amount of acid than the thymic cells. The observations on the p_H of suspensions of testicular cells of the rat and the rabbit were quite variable since these suspensions contained spermatozoa, which were not included in the count. It was not possible to compare the results with those obtained for suspensions of thymic cells.

TABLE 4.—*Decrease in the p_H of Cellular Suspensions Incubated at 45 C. for One Hour in the Presence and in the Absence of Dextrose and Oxygen. Also, the Difference in the Decrease Produced by Various Types of Cells and That Produced by Thymic Cells of the Rabbit Under the Same Conditions*

Source of Cells (Tissue and Species)	Unstained Cells per Millimicroliter	p_H of Suspension After 1 Hour of Incubation				Differences in the p_H of Suspensions of Cells Studied and of Thymic Cells of Rabbit			
		Dextrose 0.1%		No Dextrose		Dextrose 0.1%		No Dextrose	
		Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic	Anaerobic	Aerobic
Thymus									
Rabbit	37	7.26	7.29	....	....	.....	.....	.....	.....
Rabbit	92	6.90	7.22	7.30	7.45	.....	.....	.....	.....
Rabbit	240	6.38	....	7.08	....	.....	.....	.....	.....
Rat	118	6.73	7.08	7.30	7.38	—0.09	—0.08	+0.03	—0.07
Sheep	57	6.98	7.16	7.22	7.48	—0.10	—0.09	—0.14	+0.03
Lymph node									
Rabbit	73	6.71	6.95	7.28	7.39	—0.26	—0.24	—0.04	—0.06
Man	46	6.75	6.85	....	....	—0.38	—0.42*	.....	.....
Testicle									
Rabbit	57†	6.92	6.99	7.08	7.22	.....	.....	.....	.....
Rat	60†	6.76	6.75	7.05	7.15	.....	.....	.....	.....
Sheep	60§	6.52	6.61	7.10	7.22	—0.10	—0.36	—0.13	—0.13
Peritoneal fluid									
Rabbit	83	6.17	6.18	6.44	6.63	—0.80*	—1.01*	—0.88*	—0.82*
Blood									
Man	147	5.84	5.82	6.21	6.26	—0.85*	—1.23*	—1.01*	—1.17*
Lymphatic leukemia, blood									
Man	102	7.02	7.23	7.23	7.45	+0.13	+0.08	—0.06	0.00
Man	154	6.46	6.97	7.25	7.41	—0.20	—0.06	+0.04	—0.05
Man	70	6.88	7.30	7.28	7.45	—0.14	+0.08	—0.06	0.00
Myelogenous leukemia, blood									
Man	130	6.22	6.40	6.76	6.95	—0.55*	—0.60*	—0.49*	—0.50*
Man	132	6.00	6.13	6.70	6.83	—0.76*	—0.95*	—0.54*	—0.62*
Eosinophilic leukemia, blood									
Man	50	6.71	6.65	7.21	7.25	—0.40*	—0.61*	—0.18	—0.20
Giant follicular lymphoblastoma, node									
Man	95	6.41	6.76	7.10	7.38	—0.51*	—0.40*	—0.20	—0.07
Lymphatic leukemia, nodule									
Mouse	180‡	6.16	6.17	7.06	7.16	—0.35*	—0.79*	—0.10	—0.29
R39 sarcoma									
Rat	15	6.33	6.16	7.00	7.10	—0.98*	—1.18*	—0.43*	—0.35*

* The difference exceeds 0.40 and is considered significant. These cells produce more acid than the thymic cells of the rabbit under identical conditions.

† The suspensions also contained unstained spermatozoa, which are not included in the count.

§ The p_H in this suspension was determined after three hours of incubation. The suspension of thymic cells used for comparison was also incubated three hours.

‡ The concentration of dextrose in this suspension was 0.25 per cent instead of 0.1 per cent.

The cells derived from peritoneal exudates of rabbits caused a greater decrease in p_H than a similar number of thymic cells (table 4). For example, the p_H decreased to 6.17 in an anaerobic suspension containing 83 viable cells from the exudate and to 6.97 in a suspension of thymic cells. The marked difference of 0.80 is definitely significant. It was also found that the leukocytes derived from normal human blood caused a much greater decrease in the p_H of dextrose-containing suspensions than thymic cells of the rabbit. The difference (0.85) was approximately the same as that observed for cells from the peritoneal exudate of rabbits. It should be noted that the majority of cells in the suspensions derived from human blood and from the exudate were polymorphonuclear leukocytes. It seems that this type of cell has a greater capacity

than the thymic lymphocyte to produce acid in dextrose-containing suspensions incubated anaerobically.

p_H of Suspensions of Normal Cells Under Aerobic Conditions in the Presence of Dextrose.—Thymic cells of the rabbit incubated aerobically with dextrose also produced a decrease in the p_H of suspensions (table 4). The decrease was not as great as that observed under anaerobic conditions (p_H of one suspension was 7.22 after one hour of aerobic and 6.90 after one hour of anaerobic incubation). The thymic cells of the rat and the sheep confirmed this finding. It may be concluded that lymphocytes of the thymus in the presence of dextrose produce less acid aerobically than anaerobically.

Testicular cells of the rat, the rabbit and the sheep produced approximately as great a decrease in the p_H of suspensions under aerobic as under anaerobic incubation at 45 C. This type of cell apparently differed from the thymic lymphocyte in having a relatively greater capacity to produce acid under aerobic conditions.

The cells of the peritoneal exudate from rabbits and of human blood produced a considerable decrease in p_H under aerobic conditions (table 4). The decrease was as large or almost as large as that observed after anaerobic incubation (the p_H of a suspension of human blood cells decreased to 5.82 and 5.84 after one hour of aerobic and anaerobic incubation).

p_H of Suspensions of Normal Cells in the Absence of Dextrose.—In dextrose-free suspensions of thymic cells maintained under anaerobic conditions, the p_H showed a slight decrease or remained stationary (table 4). The slight decrease might be attributed to the failure to remove by washing minimal amounts of dextrose. In contrast, leukocytes derived from peritoneal exudate and from blood showed a considerable decrease in p_H in a dextrose-free medium. The decrease in p_H was not, however, as great as in suspensions containing dextrose (the cells of human blood, for example, decreased the p_H to 6.21 in a dextrose-free medium and to 5.84 in suspensions with sugar).

No change or a slight increase in the p_H was observed in dextrose-free suspensions of thymic cells incubated aerobically. In contrast, leukocytes of blood and of peritoneal exudate caused a considerable decrease, which was approximately as great as that observed in the dextrose-free medium under anaerobic conditions.

p_H of Suspensions of Cells in the Presence of Other Sugars.—The simplicity in the determination of the changes in p_H in the presence of dextrose suggested an investigation of the effect of other types of sugar. Suspensions from the thymus of the rabbit were incubated anaerobically with equal amounts of sugar–phosphate–Ringer solution. The buffer in the solution was reduced to 5 per cent. The results are presented in table 3. It is seen from the table that suspensions containing dextrose or mannose showed a marked decrease in p_H (to 6.13) after incubation for one-half hour and a further decrease (to 5.68 and 5.59) after another one-half hour. On aerobic incubation for two and one-half hours, both of these sugars caused an equal decrease in p_H to 5.92. In contrast, the other sugars were associated with no, or only minor, changes in the p_H. The most suggestive decrease was obtained with galactose; the p_H dropped from 6.96 to 6.84. It may be concluded that rabbit lymphocytes were able to ferment dextrose and mannose and, to a lesser extent, galactose. There was no perceptible fermentation of fructose, xylose, sucrose or maltose.

p_H of Suspensions of Neoplastic Cells.—A suspension from a lymph node of a patient with giant follicular lymphoblastoma had a concentration of 95 viable cells per millimicroliter. The p_H of the dextrose-containing suspension decreased moderately on anaerobic incubation (to 6.41) but showed a lesser decrease under aerobic conditions (to 6.76) and showed no significant changes in dextrose-free mediums.

It is seen from table 4 that the cells of this tumor produced a somewhat greater amount of acid in the dextrose-containing medium than the thymic cells of the rabbit (differences in p_H were 0.51 and 0.40 under anaerobic and aerobic conditions). The lymphocytes in a suspension of a hyperplastic lymph node of a patient were also found to develop a greater quantity of acid than cells of the thymus (differences in p_H were 0.38 and 0.42, respectively). It seems, then, that the reactions of the cells of giant follicular lymphoblastoma and the hyperplastic lymph node were similar.

The blood from 3 patients with chronic lymphatic leukemia was studied. The reactions were substantially the same in all the cases (table 4). A suspension of the leukocytes of 1 patient, for example, had 154 cells per millimicroliter. The p_H decreased to 6.46 and 6.97 in the dextrose-containing medium under anaerobic and aerobic conditions respectively, and did not show any appreciable changes in the absence of dextrose. It is seen from the table that the leukocytes of lymphatic leukemic blood had distinctly different physiologic reactions when compared with the cells of normal human blood but had substantially the same reactions as the lymphoctyes of the rabbit's thymus.

Blood from 2 patients with myelogenous leukemia was obtained. In 1 case a concentration of 132 viable cells per millimicroliter caused a considerable decrease in p_H (to 6.00 and 6.13) in the dextrose-containing mediums and a moderate decrease (to 6.70 and 6.83) in the dextrose-free mediums (table 4). The observed changes were not quite as large as those obtained for leukocytes of normal blood. The findings are, however, in sharp contrast with the results obtained for lymphatic leukemia.

The blood of 1 patient with eosinophilic leukemia was studied. In a suspension containing 50 cells per millimicroliter the p_H decreased moderately under anaerobic and aerobic conditions in the presence of dextrose (to 6.71 and 6.65) and showed no appreciable change in the absence of this sugar. These findings were similar to those obtained for lymphatic leukemia and were quite different from those for myelogenous leukemia.

Studies on normal and on leukemic leukocytes obtained from other patients were made at temperatures of 48, 50 and 52 C. The results are presented in part in table 5. It is seen that normal leukocytes produced the greatest changes in the p_H, myelogenous leukemic cells decreased the p_H almost as much, and the lymphatic leukemic cells caused only slight changes. The findings are in complete agreement with those obtained in the previous experiments.

A suspension derived from mice with lymphatic leukosis showed a marked decrease in the p_H of a dextrose-containing medium both anaerobically and aerobically (table 4 shows that 189 cells per millimicroliter decreased the p_H to 6.16 and 6.17, respectively) and no change in the dextrose-free mediums. Apparently the cells of this tumor produced more acid in the presence of dextrose than the lymphocytes of the thymus or those of the blood of patients with lymphatic leukemia. The suspension of the tumor of the mouse was prepared with a higher concentration of dextrose (0.25 per cent instead of 0.1 per cent), which was at least partly responsible for the greater production of acid.

R39 sarcoma produced the most marked decrease in the p_H of suspensions with dextrose. A concentration

of only 15 cells caused the p_H to fall to 6.33 anaerobically and 6.16 aerobically. No definite changes were observed in the dextrose-free suspensions.

The findings on the effect of cells on the p_H of suspensions may now be summarized. The species of animal from which the cells were obtained did not appear to be a factor in the production of acid, according to the present experiments. The decrease in p_H under aerobic and under anaerobic conditions depended primarily on the type of cell. The normal, the hyperplastic and the neoplastic lymphocytes caused (1) a moderate decrease in the p_H of the dextrose-containing medium under anaerobic incubation, (2) usually a lesser decrease after aerobic incubation and (3) no definite change in the dextrose-free medium. Normal, inflammatory and neoplastic polymorphonuclear leukocytes were characterized by (1) a striking decrease in p_H on anaerobic incubation of dextrose-containing suspensions, (2) an equally large decrease aerobically and (3) a fairly large decrease in the dextrose-free medium on aerobic and anaerobic incubation. Testicular cells produced (1) a moderate decline in p_H anaerobically in the presence of dextrose, (2) approximately the same decrease aerobically and (3) no appreciable change in p_H in the absence of sugar.

the decrease in p_H is a measure of the anaerobic or the aerobic glycolysis of the cells. If this interpretation is correct, it would seem that the present method of studying simultaneously the p_H of the medium and the number of viable cells affords a simple means of determining quantitatively the amount of aerobic or of anaerobic glycolysis per viable cell instead of per milligram of tissue.

All the cells studied were capable of producing acid in dextrose-containing mediums under anaerobic conditions. The findings suggested that different types of cells varied in their capacity to produce acid. Further studies are needed, however, to correlate the decrease in p_H with the type, the size and the physiologic activity of the cell.

All the cells studied were capable of aerobic glycolysis, i. e., of producing acid in dextrose-phosphate-Ringer solution on aerobic incubation

TABLE 5.—*Decrease in the p_H of Suspensions of Cells Obtained from Normal and from Leukemic Blood of Patients*

Source of Cells	Unstained Cells per Millimicroliter	Temperature of Incubation, C.	p_H of Suspension After 1 Hour of Incubation			
			Dextrose 0.1 per Cent		No Dextrose	
			Anaerobic	Aerobic	Anaerobic	Aerobic
Normal blood	132	50	5.88	5.76	6.72	6.68
	81	50	6.13	6.01	7.00	7.05
Blood of patient with myelogenous leukemia	144	48	6.15	6.15	6.71	6.88
	140	50	6.55	6.55	6.98	7.00
	85	50	6.40	....	6.96	6.93
	25	50	7.02	7.15	7.28	7.38
Blood of patient with lymphatic leukemia	101	52	6.80	6.75	7.21	7.30

COMMENT

It would seem desirable to conduct experiments on cellular physiology at the temperature of 37.5 C. In this preliminary survey it was found more convenient to work at 45 C., as this temperature accelerates the activities of the cells but inhibits bacterial growth. The results are of interest whether they represent, as was assumed, accelerated physiologic reactions or whether they depend on an abnormal response to an elevated temperature.

Physiology of Normal Cells.—Glycolysis, or the utilization of dextrose by cells, has been extensively studied by means of the Warburg manometric and chemical methods. The literature has been reviewed recently by Stern.[2] This previous work on glycolysis aids in the interpretation of the present observations on the changes in p_H of cellular suspensions. The decrease in p_H after aerobic and anaerobic incubation is evidently due to the fermentation of dextrose with the formation of lactic acid. It is probable that at 45 C. Different types of cells varied considerably in this capacity. Testicular cells and polymorphonuclear leukocytes produced approximately as much acid under aerobic as under anaerobic conditions. Similarly MacLeod [3] found by means of the manometric method that spermatozoa had a high capacity for aerobic glycolysis at 38 C. In contrast, the lymphocytes of the thymus had lesser capacity for aerobic than for anaerobic glycolysis.

The polymorphonuclear neutrophilic leukocyte derived from peritoneal exudate or human blood was capable of producing acid in dextrose-free mediums. Morphologic studies on this cell have shown cytoplasmic masses which on staining appear to be glycogen (Cowdry [4]). It is probable, then, that this cell, like the muscle cell, contains a reserve supply of carbohydrate.

The importance of the metabolism of dextrose in the physiologic activity of the muscle cell is well known. MacLeod [3] has shown similarly that the motility of the spermatozoa is dependent

2. Stern, K., and Willheim, R.: Biochemistry of Malignant Tumors, Brooklyn, Chemical Publishing Company, 1943.

3. MacLeod, J. O.: Human Fertil. **7**:129, 1942.

4. Cowdry, E. V.: Special Cytology; The Form and Functions of the Cell in Health and Disease, New York, Paul B. Hoeber, Inc., 1928, vol. 1, p. 408.

on glycolysis. It may be possible to correlate the motility of the neutrophilic leukocyte with the present findings of the high capacity of this cell for aerobic and anaerobic glycolysis both in the presence and in the absence of added dextrose.

Although glycolysis is important to cells as a source of energy, the present experiments showed that the presence or the absence of dextrose did not affect appreciably the in vitro survival period of the cells studied. Glycolysis, then, was not essential to the survival of cells in vitro.

A second physiologic process suitable for investigation by the manometric method is the utilization of oxygen by cells, i. e., cellular respiration. In the present experiments this process was not studied directly, but observations were made on the effect of exposure to air on the in vitro survival of cells. In an occasional experiment it was found that atmospheric oxygen appeared to be toxic to the cells. This finding is similar to MacLeod's [8] observation that spermatozoa become nonmotile when subjected to 95 per cent oxygen. MacLeod attributes this finding to the absence of catalase.

All the types of cells studied except the thymic cells of the rabbit were found to be capable of remaining viable as long in the absence of both oxygen and dextrose as in the presence of these substances. Evidently the cellular reactions of respiration and glycolysis are not essential for the survival of cells in vitro.

In all probability the survival of cells necessitates the expenditure of energy. What, then, is the chemical reaction which permits cellular survival in vitro? In the present investigation this problem was not studied, but it seems reasonable to assume that the survival of cells in vitro depends on the metabolism of proteins or nucleoproteins.

Although cells can survive under anaerobic conditions, animals can withstand the absence of air for only a few minutes. Death in these cases is usually attributed to the tissues' lack of oxygen. Cerebral cells are reputed to be particularly sensitive to this deprivation. In view of the present work the death of the animal or the injury of cerebral tissue may not be due to anoxemia but may be rather the result of the anaerobic production of lactic acid.

Physiology of Neoplastic Cells.—The methods for determining the survival of cells and for studying aerobic and anaerobic glycolysis seemed to offer a new approach for a comparative study of normal and cancerous cells. In this preliminary survey, leukemia was the most convenient neoplastic or presumptively neoplastic disease to study. The present findings suggest that the survival periods of malignant and normal homologous cells were approximately the same. The aerobic and the anaerobic glycolysis appeared to depend in part on the character of the ancestral cell and in part on the malignancy of the cell.

The cells of myelogenous and lymphatic leukemia differed considerably in their capacity to produce acid in mediums with and without dextrose. The differences were so marked that they appeared to be of value in the differential diagnosis of the two types of leukemia.

SUMMARY AND CONCLUSIONS

By the method of unstained cell counts, the survival of cells at 45 C. was determined under aerobic and under anaerobic conditions, in the presence and in the absence of dextrose. The major factor determining the length of survival was the type of cell. In most cases the survival of cells was not affected appreciably by dextrose or oxygen. Most types of cells studied were able to survive for prolonged periods in the absence of both dextrose and oxygen. In contrast, the thymic cells of the rabbit required the presence of either oxygen or a fermentable sugar for survival.

It appeared from these experiments that the survival of cells in vitro depended not on respiration or on glycolysis but on some other cellular reaction, possibly the metabolism of nucleoproteins.

Aerobic and anaerobic glycolysis of cells was studied by determining the number of viable cells and the p_H of the suspensions before and after incubation. Decrease in the p_H of the suspension depended on such factors as the number and the type of cells and the duration of incubation. In peritoneal fluid and suspensions derived from the testicle as much acid developed aerobically as anaerobically. Thymic cells produced less acid under aerobic conditions. The thymic cells of the rabbit were able to ferment dextrose and mannose and possibly galactose but not fructose, xylose, sucrose and maltose. Polymorphonuclear leukocytes of peritoneal exudate and of human blood produced acid in a dextrose-free medium, apparently by utilization of glycogen in the cells.

Leukemic cells derived from patients were able to survive as long in vitro as the normal leukocytes of blood. Myelogenous and lymphatic leukemic cells differed markedly in their capacity for aerobic and anaerobic glycolysis.

Case Reports

TRUNCUS ARTERIOSUS COMMUNIS PERSISTENS

CAPTAIN OSCAR B. HUNTER JR.
MEDICAL CORPS, ARMY OF THE UNITED STATES

In 1910 Herxheimer [1] reported 43 cases of truncus arteriosus communis persistens. Abbott [2] analyzed 21 cases of persistent common arterial trunk out of a group of 1,000 cases of congenital heart disease. Humphreys [3] reviewed the literature and established criteria for the identification of a persistent common trunk and listed differences from solitary pulmonary and aortic trunks. Her criteria were these: (1) four cusps in the semilunar valve; (2) coronary arteries arising from the common trunk; (3) pulmonary arteries arising from the common trunk; (4) systemic vessels arising from the common trunk; (5) absence of structures derived from the sixth arch. According to her review 15 cases could be established as instances of true common trunk from among those reported in the literature. Lev and Saphir [4] reviewed all cases reported since 1932 and found 12 definite and 7 possible cases. Their criteria were essentially the same, but they rejected the necessity of four cusps, stating that although the embryologic conditions required these, primary anomalies of these valves do occur. In this they concurred with Abbott and many of the German authors.

Since January 1942 2 other cases have been reported. Van Brown [5] reported the case of an 11 month old boy who died as the result of this anomaly. The heart in his case gave rise to a persistent trunk from the right ventricle. The vessel had four semilunar cusps guarding its orifice. The coronary arteries arose from a right and a left lateral sinus. Above the valve one large arterial trunk emerged which divided into pulmonary and aortic portions, the division being constituted by an archlike ridge. The pulmonary and aortic divisions were transposed, the pulmonary artery dividing shortly into a right and a left branch. A partially obliterated ductus arteriosus was present. Above the pulmonary artery the innominate, the left common carotid and the left subclavian artery arose in that order from the arch. The aortic end of the duct was funnel shaped and in its usual position.

This is classified as a "partial" defect. It conforms to the true anomalous development of the persistent trunk by the presence of four cusps from the semilunar valve and also by the fact that the coronary, pulmonary and systemic vessels arise from the common trunk.

The other case was described by Doerr [6] which apparently was one with transposition of the pulmonary and aortic sections from the common trunk. The literature on this, however, is not available.

REPORT OF A CASE

Aug. 11, 1943 a 6 month old boy was admitted to the Borden General Hospital because of cyanosis associated with dyspnea, grunting, rapid respiration and fever. The child was born one month prematurely by a normal spontaneous delivery and weighed 7½ pounds (3,402 Gm.). Cyanosis of the finger nails, toe nails and circumoral region were noticed at birth. Two weeks after birth a congenital indirect complete inguinal hernia became evident, and at 6 weeks of age hernioplasty was performed. His mother noticed generalized cyanosis associated with crying, straining at stools and like exertions. These spells would last from a few minutes to several hours.

There were marked general cyanosis, dyspnea, expiratory grunt, tachycardia and dehydration. The mucous membranes were purplish red. The lungs were normal to percussion and auscultation. The heart was displaced to the right, with the maximum impulse at the right lower border of the sternum. The rate was extremely rapid. There were a loud systolic murmur at the lower right border of the sternum and a soft to and fro murmur at the base. The abdomen was distended. The edge of the liver was palpable. Hypospadias, first degree, was noted. Moderate clubbing of the fingers and toes was present.

There was leukocytosis (white cell count, 27,000 cells per cubic millimeter, with 80 per cent polymorphonuclear leukocytes). The red cell count was 4,600,000, with 8 per cent nucleated red cells, and 15.2 Gm. of hemoglobin per hundred cubic centimeters of blood. The electrocardiogram revealed right axis deviation and marked sinus tachycardia. Roentgen examination of the chest showed the trachea to be deviated markedly to the right, with apparent displacement of the mediastinum into the upper portion of the right side of the chest. The heart showed moderate uniform enlargement.

The infant responded well to oxygen therapy and parenterally administered fluids. On the second hos-

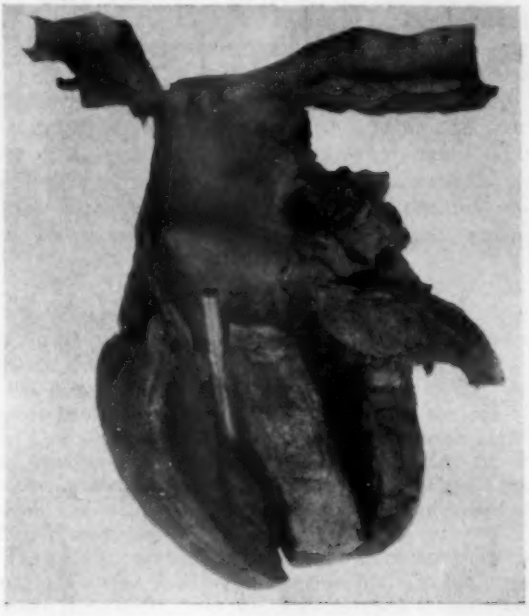
From the Borden General Hospital, Chickasha, Okla.

1. Herxheimer, G.: Missbildungen des Herzens und der grossen Gefässe, in Schwalbe, E.: Die Morphologie der Missbildungen des Menschen und der Tiere, Jena, Gustav Fischer, 1910, p. 427; cited by Humphreys.[3]
2. Abbott, M. E.: Atlas of Congenital Cardiac Disease, New York, American Heart Association, 1936.
3. Humphreys, E. M.: Arch. Path. **14**:671, 1932.
4. Lev. M., and Saphir, O.: J. Pediat. **20**:74, 1942.
5. Van Brown, D.: J. Tech. Methods **22**:101, 1942.
6. Doerr, W.: Virchows Arch. f. path. Anat. **310**: 304, 1943.

pital day signs of pneumonia of the entire right lung developed. This cleared under oxygen and sulfadiazine therapy. On the tenth day the child became restless and cyanosis increased. From this time on the course was downhill, and death occurred August 22.

Postmortem examination revealed truncus arteriosus communis persistens with a patent foramen ovale, an interventricular septal defect and patency of the ductus arteriosus giving rise to the pulmonary arteries. Atelectasis and hemorrhagic edema of the right lung; hyperplastic bone marrow; fatty changes in the liver, myocardosis and interstitial fibrosis of the pancreas; multiple pelves in both kidneys; right renal artery arising from the right common iliac artery and the right kidney lying on the brim of the pelvis; grade 1 hypospadias.

The heart lay in approximately the center of the thorax. The pericardium measured transversely 7.5 cm.



Persistent truncus arteriosus communis: Anterior view of the heart showing opening of the left ventricle into the trunk. A probe is inserted from the trunk into the right ventricle. (Photograph made by the United States Army Medical Museum, neg. no. 77202, acc. no. 98855.)

The right border was 3.5 cm. from the midline. The left border was 4 cm. from the midline. The transthoracic diameter was 11 cm. The heart was estimated to weigh 50 Gm. The color of the musculature was dull red, and the consistency was normal. When the heart was opened, there was seen to be a congenital malformation of the chambers. The right atrium was markedly dilated and contained approximately 3 cc. of blood. The tricuspid valve measured 4.5 cm. in circumference and had three normal-appearing cusps. The right ventricle contained approximately 2 cc. of fluid. The depth of the right ventricle was 3 cm., and the thickness of the wall of the right ventricle was 8 mm. The left atrium contained approximately 1 cc. of blood and was connected with the pulmonary veins from both sides. There was a patent foramen ovale which allowed the passage of a probe 3 mm. in diameter. The mitral valve was 1.6 cm. in diameter and had two normal-appearing cusps. The left ventricle measured 3 cm. in depth, and the thickness of the wall was 8 mm. The aorta and the pulmonary arteries were undifferentiated, thus forming a persistent arterial trunk. The mouth of the vessel was 4 cm. in circumference. The orifice was guarded by three well developed cusps. The right coronary artery arose from the anterior sinus, and the left coronary artery arose from the left posterior sinus. The right posterior sinus gave rise to no vessel. The interventricular septum was defective at its membranous upper edge. The septum lay immediately beneath the center of the common trunk and consequently blood from both sides of the heart apparently mixed equally in the trunk. The myocardial fibers of the ventricles seemed to terminate in a fibrous ring which encircled the great vessel. In the common trunk itself there was no evidence of any septum or of any anlage of one. The trunk gave rise to the two coronary arteries, an innominate artery, the left common carotid artery and the left subclavian artery, in that order. On the descending portion of the arch the ductus arteriosus, in the usual position, was seen to be patent and measured 6 mm. in diameter. This vessel gave rise to a right pulmonary artery, which measured 2 mm. in diameter. The right pulmonary artery coursed across the mediastinum and entered the hilus of the right lung in the normal position and relation. Immediately before entering the hilus it gave rise to the left pulmonary artery, which measured 2 mm. in diameter. This vessel retraced the course of the right pulmonary artery and entered the left hilus in the normal position and relation. Diligent search failed to demonstrate any other vessel arising from the base of the heart. Between the base of the heart and the ductus arteriosus there was definitely no patent vessel, and as far as it was possible to determine there was no evidence of any fibrous cord, the remnant of an atretic vessel.

The remainder of the vascular branches of the aorta in the thorax did not appear unusual. In the abdomen the left renal artery arose in normal relationship 1 cm. below the celiac axis. The right renal artery arose from the right common iliac artery in its first few millimeters.

COMMENT

Herxheimer [1] reported 43 cases of truncus arteriosus communis persistens but was found by later authors to have included cases of atresia of the aorta and the pulmonary arteries. Even Abbott's [2] compilation of cases contained a few of these instances of atresia. Humphreys [3] was the first to establish definite criteria for the diagnosis of persistent common arterial trunk. Her review lists 15 unquestionable cases including 1 of her own. Her criterion requiring four cusps for the semilunar valve does not allow for the possibility of additional anomalies of the anlage of these cusps. The completeness of her investigation of the embryologic development of these cases established a definite starting point. The criteria of Lev and Saphir [4] rejected the necessity of four cusps and required only that the large vessel give rise to the coronary, pulmonary and systemic vessels. The cases reported by Van

Brown [3] and by Doerr [6] are apparently true cases of persistent trunk and both apparently belong to the group of "partial" trunk, i. e., having a ridge separating the pulmonary branch from the aortic branch.

The case now reported is the thirty-seventh and is distinctly different from the previous ones. It simulates many of them in that the truncus is in the "rider" position, i. e., straddling both ventricles. The anomaly is unusual in that the pulmonary arteries arise only from a large vessel which branches from the truncus in the position of the ductus arteriosus. Careful examination eliminated the possibility of an atretic pulmonary vessel. The only source for the pulmonary arteries was the ductus arteriosus. The type of deformity is "complete," there being no evidence of any ridge or septum in the wall of the truncus. From the position of the coronary arteries it would be presumed that torsion had taken place, but the position of the vessels gives no evidence of any simulation of transposition, although the place of the heart in the body on the right side suggests that no torsion had taken place.

SUMMARY

The case of truncus arteriosus communis persistens reported is the thirty-seventh of its type and the first with the pulmonary arteries arising from a patent ductus arteriosus.

GENERAL NEUROFIBROMATOSIS (VON RECKLINGHAUSEN'S DISEASE) WITH LOCAL SARCOMATOUS CHANGE AND METASTASIS TO REGIONAL LYMPH NODES

M. WACHSTEIN, M.D., MIDDLETOWN, N. Y., AND E. WOLF, M.D., PASSAIC, N. J.

Since Von Recklinghausen[1] first described general neurofibromatosis in 1882, many authors have contributed to knowledge of this disease. The remarkable coincidence of general neurofibromatosis with other congenital pathologic conditions (e. g., pigmentary anomalies of the skin), the changes in bones and the concomitant involvement of the central nervous system, as well as the frequent mental retardation and the hereditary character, have been stressed.[2]

The most serious complication in general neurofibromatosis is sarcomatous change of one or more of the tumors. Hosoi,[3] reviewing the literature up to 1931, collected 65 cases of sarcomatous transformation, representing 13 per cent of all published cases up to that date. We shall here report a case of neurofibromatosis in which sarcoma developed in one of the tumors and metastasized to the regional lymph nodes.

REPORT OF A CASE

The patient was a 47 year old Negro, married, who until eight years before examination had worked as a garbage collector.

The father died of apoplexy at the age of 63; the mother died of tuberculosis of the lungs at the age of 32. One sister died of tuberculosis. One brother and one sister were living. The mother had many tumor nodules scattered over the skin, similar to those of the patient. None of the other siblings showed signs of neurofibromatosis.

The patient had never had any illness except chickenpox. He stated that the nodules on his skin had been present since birth. He was first admitted to the Beth Israel Hospital in Passaic, N. J., in September 1936. He complained of pains in all his joints. There were painful swellings in most of the joints of the extremities, which subsided only after he had made a protracted stay in the hospital. The Wassermann reactions of the blood and the spinal fluid at that time were negative. Ever since that febrile episode, the patient had had pains in his left leg, starting in the back and radiating down to the foot. The pains grew worse when he walked. He also started to limp considerably. Since that time he had been an invalid, unable to work.

During the year prior to his second hospitalization he noticed that one lump on the outer surface of the left thigh, above the knee joint, had increased in size and become quite sensitive to the touch. A few weeks before his admission he noted a painless swelling in the region of the left groin. He stated that all the other cutaneous nodules had not changed in size since he could remember.

The patient was a well nourished Negro in good general condition. His mental age corresponded roughly to that of an 11 year old child. Marked kyphoscoliosis was present. The entire body was covered with innumerable small and large nodules, some of which were of pendulant character. Most of these nodules were found on the chest and the back. In addition, the skin presented small and large irregularly shaped areas of darker pigmentation. An approximately egg-sized subcutaneous nodule was found on the outer aspect of the thigh above the knee joint; the covering skin showed no abnormality. There was definite pain to the touch. Two enlarged lymph nodes were felt in the left groin. Apart from a fairly loud systolic murmur over the aortic region of the heart and blood pressure of 170 systolic and 100 diastolic, the examination of the internal organs revealed no significant changes. The right pupil was not quite round but reacted well to light and convergence. The fundi and the visual fields were normal. There was spastic paresis of the left leg with pronounced hyperreflexia and ankle clonus and with positive Babinski and Oppenheim reflexes. The left leg showed moderate atrophy of the muscles; no sensory involvement was found.

The urine showed no abnormality; the blood count was normal; the calcium in the blood amounted to 9 mg. per hundred cubic centimeters; the inorganic phosphate in the blood, to 4 mg. per hundred cubic centimeters; alkaline phosphatase was estimated at 2 Bodansky units; the Wassermann reaction of the blood was negative. The spinal fluid showed three cells per cubic millimeter; there was no increase in albumin and globulin; the total protein amounted to 16 mg. per hundred cubic centimeters, and the Wassermann reaction was strongly positive.

Roentgenographic examination (Dr. E. Schlossberg) showed the lung fields to be clear. There was moderate left ventricular enlargement with moderate widening of the aortic arch. The skull showed no bony abnormality. Several sharply circumscribed rounded dense shadows, representing neurofibromatous nodules of the skin, were visualized. The dorsolumbar region of the spinal column showed marked scoliosis; there was concavity to the left in the lumbar region, as well as hypertrophic arthritic changes. Numerous rounded, sharply circumscribed small and large dense shadows were scattered throughout the abdomen on both sides, representing neurofibromatous nodules in the skin. The femurs showed slight lateral bowing. At the medial aspect of the lower half of the left femoral shaft was a thick layer of periosteal bone formation with a smooth wavy contour. A similar smooth layer of periosteal bone formation was seen at the lateral aspect of the right tibia, involving the proximal part of the shaft.

The large subcutaneous tumor above the left knee joint was removed, and the two enlarged lymph nodes in the groin were excised several days later.

From the Laboratories of the Beth Israel Hospital, Passaic, N. J.

1. von Recklinghausen, F.: Ueber die multiplen Fibrome der Haut und ihre Beziehung zu den multiplen Neuromen, Berlin, A. Hirschwald, 1882.

2. Wilson, S. K.: Neurology, edited by A. N. Bruce, Baltimore, Williams & Wilkins Company, 1940.

3. Hosoi, K.: Arch. Surg. **22**:258, 1931.

The first specimen (fig. 1) consisted of a well encapsulated subcutaneous tumor measuring 5.5 by 4 by 3.8 cm. A segment of skin, measuring 4.5 by 4 cm., was attached to the specimen. The cut section presented a whirl-like, white, firm appearance with several small and large grayish areas. The second specimen consisted of two enlarged lymph nodes, measuring 5 by 3.8 by 2 cm. and 3.5 by 2 cm. On cut section both lymph nodes presented a similar picture. They had a grayish and whitish, cellular, fairly firm appearance. Tiny yellowish necrotic flecks were scattered over the cut surface. The capsules of the lymph nodes appeared to be intact.

Microscopic sections were stained with hematoxylin-eosin, Masson's trichrome stain, Heidenhain's aniline blue, Van Gieson's stain, Mallory's lead-hematoxylin stain for axis-cylinders and Bodian's method for nerve fibers. Sections through the subcutaneous tumor (fig. 2*A*) showed large areas consisting of scanty elongated cells separated by a large amount of wavy fibrils. In other fields, however, the tumor appeared to be very cellular. There was no sharp demarcation between many of these cells, the elongated ends of which frequently communicated. The nuclei were irregular and hyperchromatic. The chromatin was arranged in small clumps, and in many of the nuclei nucleoli were quite prominent. A considerable number of atypical mitotic figures were seen. Occasional giant cells, with several nuclei, were present. There was a varying amount of fibrillar substance in these cellular areas. No distinct palisading was noted in any portion of the tumor. The blood vessels were not prominent. Sections through the lymph nodes (fig. 2*B*) showed almost complete replacement by tumor tissue. Remnants of the lymphatic tissue were present only near the capsule. The cells were similar to those seen in the primary growth. However, the lesions appeared to be somewhat more cellular, and areas of degeneration were more prominent.

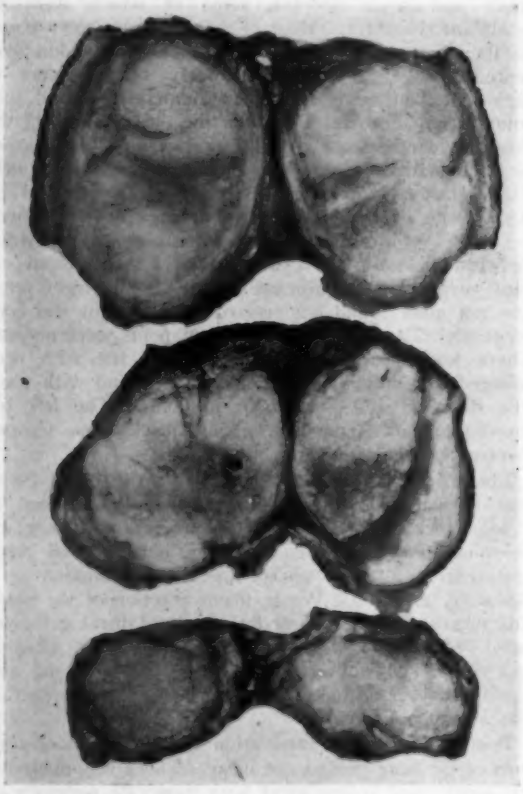


Fig. 1.—Uppermost is the subcutaneous tumor; below are the metastatically involved lymph nodes.

Masson's stain, as well as the other specific stains, revealed in the nonsarcomatous portions of the tumor large amounts of connective tissue. Connective tissue was also identified in varying amounts in the sarcomatous portions of the original tumor, as well as in the metastatically involved lymph nodes. Nerve fibers could not be demonstrated.

COMMENT

The patient presented the classic picture of general neurofibromatosis with innumerable tumors and the typical pigmentary anomalies of the skin. A hereditary factor and likewise mental retardation were present. The interpretation of the neurologic observations and of the roentgenographic findings in the skeleton is difficult, since the syphilitic infection, indicated by the positive Wassermann reaction of the spinal fluid, may have played an important etiologic role in these changes. It is worth mentioning, however, that changes in the skeleton, first described by Brooks and Lehman,[4] are estimated to occur in about 7 per cent of all cases of neurofibromatosis.[5] Scoliosis is one of the most frequent manifestations of the bone changes. Although thickening of the periosteum is most commonly encountered in syphilis, Weber[6] described a case of general neurofibromatosis with marked periosteal thickening of the left tibia.

Histologic examination of the subcutaneous tumor in areas without secondary sarcomatous change showed typical structure. It is not intended to discuss the nature of the tumor-forming cells. A discussion of this still unsettled question (Schwann cells or fibroblasts) can be found in the recent paper of Grill and Kuzma.[7] The appearance of the sarcomatous areas with their abundance of irregular cells, atypical mitotic figures and tumor giant cells has been extensively described in neurofibrosarcoma (Stewart and Copeland[8]). A careful search, of our sections failed to reveal any evidence of cross striation, so that any confusion with rhabdomyosarcoma appears to be excluded.

Metastases originating in sarcomatous neurofibroma are comparatively rare. The majority of patients suffering from neurosarcoma if not treated succumb to the unlimited local growth or to general cachexia. Hosoi found metastases in only 14, or 22 per cent, of the 65 reviewed

4. Brooks, B., and Lehman, E. P.: Surg., Gynec. & Obst. **38**:587, 1924.

5. Hodges, P. C.; Phemister, D. B., and Brunschwig, A.: The Roentgen Ray Diagnosis of Diseases of Bones and Joints, New York, Thos. Nelson & Sons, 1938.

6. Weber, P.: Quart. J. Med. **23**:150, 1930.

7. Grill, J., and Kuzma, J. F.: Arch. Path. **34**:902, 1942.

8. Stewart, F. W., and Copeland, M. M.: Am. J. Cancer **15**:1235, 1931.

cases. Most frequently involved are the lungs. Metastases to lymph nodes are extremely rare.[9]

We have been able to find in the literature reports of 2 cases in which involvement of lymph nodes was observed. Rose,[10] in 1884, reported a case of general neurofibromatosis in which a large sarcoma of the crural nerve was present. One paravertebral lymph node was found metastatically involved. Charache,[9b] reporting 10 cases of neurosarcoma in 1938, mentioned a case of general neurofibromatosis with sarcoma and metastatic involvement of the retroperitoneal lymph nodes. To our knowledge there is no case on record in which extensive involvement of regional lymph nodes originating in sarcomatous neurofibroma was found during life.

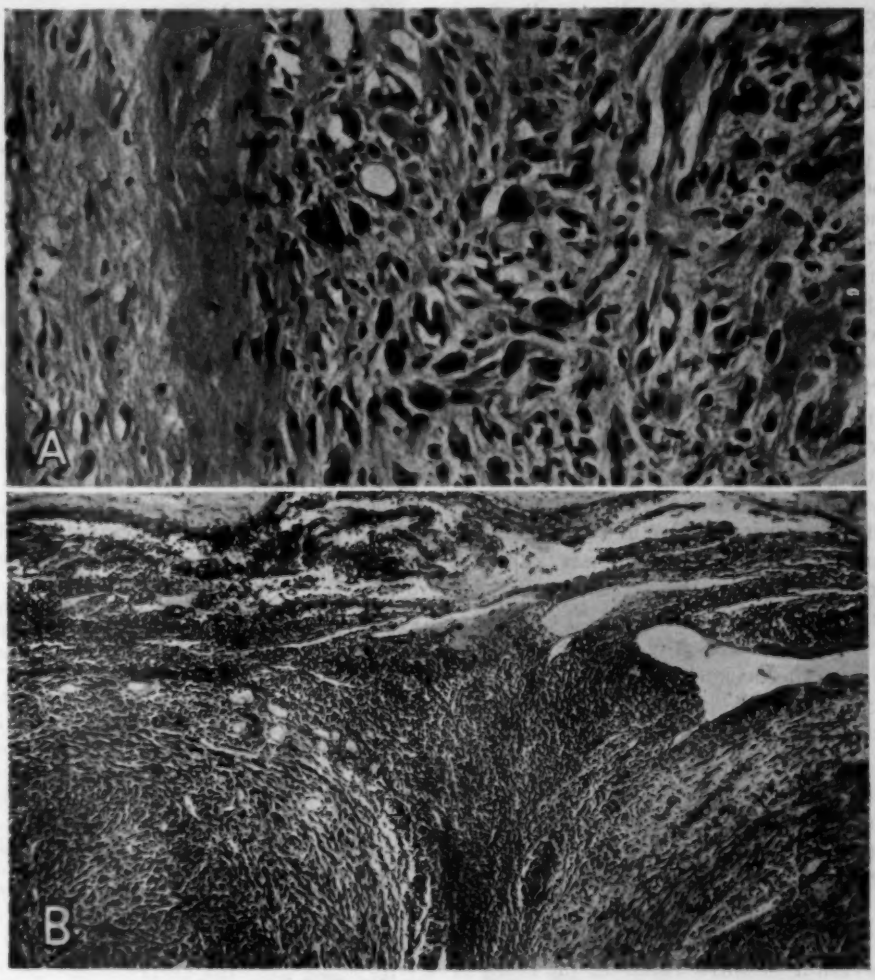


Fig. 2.—*A*, section through the subcutaneous tumor (× 225). To the left side a fibromatous area is seen; the right side shows a sarcomatous area.

B, section through a metastatically involved lymph node (× 52). Note the extensive replacement of the node by neurofibrosarcoma.

SUMMARY

A case of general neurofibromatosis with sarcomatous transformation in one of the tumors is presented. The case is remarkable because of extensive metastasis of the sarcoma to the regional lymph nodes.

9. (*a*) Willis, R.: The Spread of Tumors in the Human Body, London, J. & A. Churchill, Ltd., 1934. (*b*) Charache, H.: Am. J. Surg. **41**:275, 1938. (*c*) Stewart and Copeland.[8]

10. Rose, E.: Deutsche Ztschr. f. Chir. **19**:24, 1884; **24**:392, 1886.

PNEUMOTHORAX DUE TO METASTATIC SARCOMA

Report of Two Cases

T. F. Thornton Jr., M.D., and Robert R. Bigelow, M.D.
CHICAGO

Spontaneous pneumothorax is usually considered to be a complication of pulmonary tuberculosis.[1] The majority of patients with spontaneous pneumothorax either have clinical findings of tuberculosis or subsequently present them, but pneumothorax has been observed to develop spontaneously in the course of many other pulmonary diseases or in the absence of disease of the lung.[2] There are two modes of origin: First, there is actual perforation of the lung into the free pleural space. This happens not infrequently in the presence of cavitary tuberculosis, cysts and abscseses. In the last instance infection complicates the picture. Second, air from the lung frequently enters the pleural cavity without a tear in the pleura over the lung. Until recently this was not appreciated. Macklin [3] gave a convincing experimental demonstration of the mechanism. He found that if the intrabronchial pressure is elevated, alveoli may rupture; air then dissects along the bronchovascular sheaths to the hilus of the lung and the mediastinum. Once air reaches the mediastinum, it may extend into the neck (seen clinically as subcutaneous emphysema) or it may pass through a weak spot in the inferior portion of the mediastinal pleura to cause pneumothorax. This study offered an adequate explanation for the development of subcutaneous emphysema in the absence of pneumothorax. It also demonstrated that pneumothorax may occur in diseases of the lung and of the upper respiratory tract or in the absence of parenchymal disease of the lung if sufficient elevation of intrabronchial pressure is obtained.

Spontaneous pneumothorax in the presence of a primary neoplasm of the lung is very uncommon. It is extremely rare in the presence of a metastatic tumor of the lung; we have been unable to find a case of the latter type reported in the recent American and English literature. We wish to report 2 cases of spontaneous pneumothorax due to metastatic sarcoma.

From the Department of Surgery of the University of Chicago.

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1. Tyson, M. D., and Crandall, W. B.: J. Thoracic Surg. **10**:566, 1941.
2. Wilson, J. L.: Internat. Clin. **1**:157, 1937.
3. Macklin, C. C.: Arch. Int. Med. **64**:913, 1939.

REPORT OF CASES

Case 1.—A 19 year old white youth was first seen in the surgical clinic of the University Clinics, Chicago, July 14, 1943. Six years previously he noticed a painful swelling over the palmar surface of the metacarpophalangeal joint of the left thumb. The swelling gradually increased in size and became more painful. Six months passed before a physician was consulted, and then a local excision of the lesion was performed. The wound healed promptly, but the patient had palsy referable to the median nerve branches to the flexor, abductor and opponens muscles of the thumb following the operation. Over a two year period various exercises and treatments failed to restore function to the thumb. A course of roentgen therapy was given. By November 1942 the tumor had reappeared, with a recurrence of pain. The lesion was again excised. The wound failed to heal completely after this operation; at intervals it would break open and discharge bloody or straw-colored fluid. Two weeks before admission the pain and swelling had become aggravated.

Physical examination gave entirely negative results except for the condition of the left hand. The left thumb was swollen, indurated and tender to pressure over the metacarpophalangeal joint. The skin was thin, tense, warm and discolored. There was an operative scar along the palmar aspect of the thumb with a crusted area distal to the thenar eminence. There was no active motion of the thumb, and the index finger was somewhat atrophic. The epitrochlear and axillary nodes were not palpable.

Roentgen studies of the left hand showed osteoporosis. There was a soft tissue mass on the palmar aspect of the thumb that contained areas of calcification. The clinical impression was chondrosarcoma of the flexor tendons of the thumb. A roentgenogram of the chest was normal. Before operation the temperature rose to 99.6 F. daily.

At operation, July 17, a biopsy of the lesion was made. A frozen section was typical for fibrosarcoma. A midforearm flap amputation of the hand and removal of the axillary nodes were performed. Recovery was rapid and uncomplicated, and he was discharged on the ninth postoperative day. Dissection of the operative specimen revealed a soft, necrotic gray tumor arising from the tendon sheath of the flexor pollicis longus muscle. Microscopic sections were typical of spindle cell fibrosarcoma. The axillary lymph nodes were not involved.

The patient was seen subsequently at regular intervals in the outpatient department. He appeared to be enjoying excellent health and showed no clinical evidence of recurrence. He was readmitted to the hospital December 31, acutely ill, with the history that two weeks previously he had had a chill, followed by an infection of the upper respiratory tract. On

December 22 he suddenly became short of breath, experienced severe pleuritic pains, particularly on the right side, and began to cough with production of bloody sputum. He tried to continue at his work but soon became so fatigued and dyspneic that he had to remain in bed.

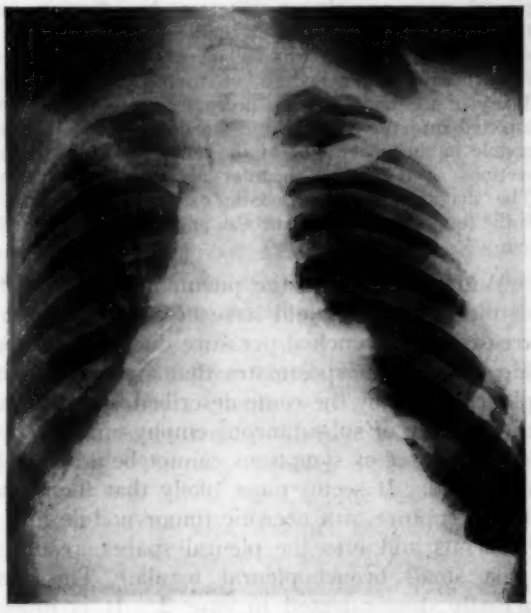

Fig. 1 (case 1).—Roentgenogram taken Dec. 31, 1943. The right lung is completely collapsed, and irregular rounded tumor nodules project from the collapsed area. The left lung shows multiple small metastatic foci, particularly in the base.

The patient appeared to be suffering considerable pain and was, as stated, acutely ill. Respirations were rapid and shallow. The chest gave increased resonance with decrease to absence of breath sounds and tactile fremitus over the right side of the chest. There was no mediastinal shift. Respirations were 34 per minute, the pulse rate 106 per minute and the temperature 100 F. The operative wounds were well healed, and there was no evidence of recurrence in the arm. There were no other positive findings.

The hemoglobin content was 17 Gm. per hundred cubic centimeters; the red blood cell count was 6,200,000 and the white cell count 12,600. A roentgenogram of the chest revealed extensive metastases to both lungs, with pneumothorax on the right side (fig. 1).

Oxygen was given at intervals by nasotracheal intubation, morphine was administered freely to relieve discomfort and anxiety and fluids were given to relieve dehydration. There was no active treatment of the pneumothorax since there was no mediastinal shift and the prognosis was hopeless. During the next week the respiration varied between 20 and 36 per minute; the pulse rate remained between 100 and 140 per minute; the temperature varied from 100 to 102 F.

Jan. 7, 1944 he suddenly became dyspneic and cyanotic and lost consciousness. Oxygen was administered by nasotracheal intubation, but the patient failed rapidly, had a short convulsion and died twenty-five minutes after the sudden onset of dyspnea. The clinical impression was that pneumothorax had developed on the left side.

Autopsy (three hours post mortem).—The pleural cavities were opened under water. Air under pressure escaped from both pleural cavities. Both lungs were completely collapsed and almost solid with firm tumor nodules. Thin bloody fluid was present in both pleural cavities in the amount of 1,200 cc. Microscopic sections of the pulmonary nodules were typical for fibrosarcoma. There were no metastases to other organs and no other significant findings. The pathologic diagnosis was extensive metastatic fibrosarcoma of both lungs with bilateral spontaneous pneumothorax.

CASE 2.—A 20 year old white youth was first seen in the orthopedic department of the University Clinics March 18, 1938. He stated that January 14 he injured his left thigh while playing basketball. Despite a great deal of pain and limp, he continued to be active. Following this injury, he began to have sharp stabbing pains in the left knee and thigh at night. About two weeks after the injury a soft swelling was noticed on the inner aspect of the knee joint. The pain in the knee and the swelling both became more severe. One week before he entered the clinic, swelling and pain in the region of the hip joint appeared. He had lost 15 pounds (6.8 Kg.) in the course of his illness.

Positive physical findings were limited to the left lower extremity. There was a swelling over the lateral aspect of the left thigh at the level of the greater trochanter. This area was not tender, warm or reddened. Just above the patella on the anteromesial aspect of the thigh was a firm fusiform swelling that was tender to deep palpation and definitely warm. The patient was immediately admitted to the hospital, and roentgenograms of the left femur demonstrated a lesion,

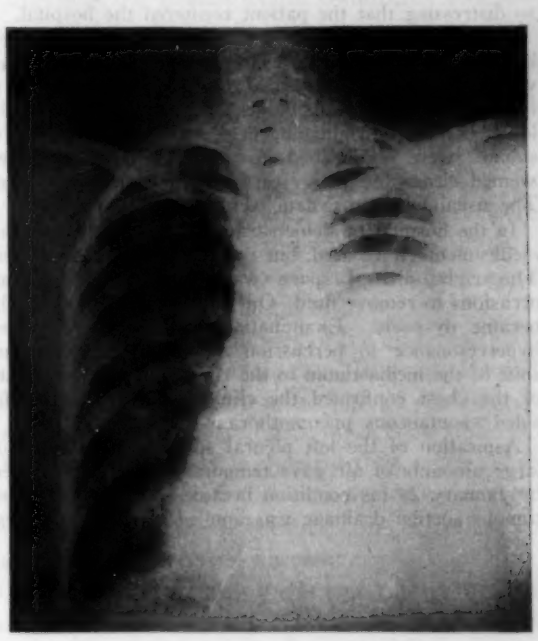

Fig. 2 (case 2).—Roentgenogram taken Jan. 26, 1939, three days before death. The hydrothorax on the right has increased despite thoracenteses, and pneumothorax is present on the left side.

6 by 8 cm., arising about 10 cm. from the distal end of the bone. Periosteal new bone was evident. The roentgen films of the chest revealed no evidence of intrinsic pulmonary disease or neoplasm. The clinical impression was osteogenic sarcoma of the lower end

of the femur. Amputation was advised, and a hip joint disarticulation of the left leg was done the following day (March 19). Dissection of the specimen disclosed a large tumor, approximately 15 cm. long, in the midportion of the femur. Microscopic sections were typical for osteogenic sarcoma.

The reaction to the operation was severe. On the first postoperative day the temperature was 104 F. and the pulse rate 140 per minute. By March 21 definite crepitus was elicited over the left side from the operative site up to the ribs, and a large area of necrosis had developed in the anterior flap. Cultures of material from the wound made at this time were positive for Clostridium welchii. Gas gangrene antitoxin, sulfanilamide, blood transfusions and parenteral fluids were given. There was marked improvement in the general condition, and the temperature and the pulse rate gradually returned to normal. A large portion of the anterior flap was removed, and the resulting ulcer closed slowly. It was not healed completely until November 12.

Routine visits were made to the outpatient clinic at weekly intervals. August 27 a roentgenogram of the chest demonstrated small metastatic lesions in both lungs. Roentgen therapy was given in the amount of 1,239 r to each of four portals over the lungs. The metastatic foci became more numerous, and roentgen therapy was repeated to the extent of another 738 r to the right lung and 492 r to the left lung. By November 25 hydrothorax had developed in the right pleural space. Thoracentesis was performed on three occasions and a total of 1,130 cc. of thin blood-tinged fluid removed. He began to have a dry cough, pain in the chest, dyspnea and anorexia. By December 10 these symptoms were so distressing that the patient reentered the hospital.

There was a well healed scar at the site of the left hip joint disarticulation. The general nutritional state was excellent, and abnormal findings were limited to the chest. There was dulness to percussion over the lower half of the right lung field, and in this area breath sounds were diminished to absent. The left lung seemed clear, and there were no rales or friction rub. The usual laboratory data were normal.

In the hospital he demonstrated little change. Various medicaments were used, but the cough was not relieved. The right pleural space was aspirated on various occasions to remove fluid. On Jan. 26, 1939 he suddenly became dyspneic. Examination of the chest revealed hyperresonance to percussion over the left lung and shift of the mediastinum to the right. A roentgenogram of the chest confirmed the clinical impression of left-sided spontaneous pneumothorax (fig. 2).

Aspiration of the left pleural space with removal of large amounts of air gave temporary relief. However, by January 28 his condition became so grave that continuous suction drainage was applied to the left pleural space. Despite efforts to keep the left lung expanded, he became rapidly worse and died on January 29.

Necropsy (six hours post mortem; Dr. W. A. Stryker).—There were no significant findings except for the scars at the site of amputation and the conditions in the chest. No air escaped when the left pleural cavity was opened under water. The right pleural space was almost obliterated by adhesions, and there was 100 cc. of clear fluid in the left pleural space. The left lung was collapsed. Both lungs were riddled with tumor nodules up to 3 cm. in diameter. Many of the nodules were necrotic, and when air was injected into the trachea it escaped through a necrotic nodule in the upper lobe of the left lung. Microscopic sections of the tumor nodules revealed osteosarcoma. The diagnosis was metastatic osteogenic sarcoma in both lungs with spontaneous pneumothorax on the left.

Without question, the pneumothorax in both of these patients could have been caused by increased intrabronchial pressure due to coughing. However, if one postulates that air reached the pleural space by the route described by Macklin,[3] the absence of subcutaneous emphysema and the sudden onset of symptoms cannot be adequately explained. It seems more likely that there was actual rupture of a necrotic tumor nodule into a bronchus and into the pleural spaces, resulting in a small bronchopleural fistula. This was actually demonstrated in case 2. It is indeed striking that spontaneous pneumothorax does not occur more frequently in the presence of metastatic tumors of the lungs. Metastases are usually multiple and often attain considerable size before death. Necrosis is a prominent feature in these tumor nodules, and one might expect that perforation into the pleural space would be commonplace. However, the fact that metastatic tumors usually do not involve a bronchus may explain the rarity of pneumothorax complicating cases of pulmonary metastasis.

SUMMARY

Pneumothorax as a complication of a case of metastatic tumor of the lung is rare, as evidenced by the lack of reports of such an occurrence in the recent literature. In the 2 cases reported here spontaneous pneumothorax was due to metastatic sarcoma.

Laboratory Methods and Technical Notes

A NEW METHOD OF FEEDING CHOLESTEROL TO ANIMALS

O. J. Pollak, M.D., Taunton, Mass.

Feeding of cholesterol as a means of producing experimental atherosclerosis has been widely used ever since it was introduced by Anitschkow and Chalatow.[1] However, their method has many disadvantages. The usual amount of cholesterol used for a single feeding of a rabbit is 0.5 Gm. Ten cubic centimeters of sunflower or cottonseed oil is required to dissolve this amount, a volume of oil which is relatively large for a rabbit. Diarrhea, which is often fatal, is largely due to the great quantity of oil, as can be shown by the equal incidence of this condition in series of animals fed oil without or with added cholesterol. The oily solution of cholesterol is stable only at 35 to 40 C., and the tube and syringe must be kept warm. In rabbits, lipid pneumonia is produced when the stomach tube slips accidentally into the trachea. (In guinea pigs, traumatic perforating ulcers of the esophagus are encountered.) The procedure is time consuming, and skill and experience are required to administer satisfactorily equal volumes at all feedings. For all these reasons large control series of animals, fed oil without added cholesterol, must be set up.

Realizing the disadvantages of the tube feeding method, Weinhouse and Hirsch[2] fed their rabbits 1 Gm. of cholesterol mixed into Purina rabbit chow. The cholesterol must be dissolved in ether and mixed with a weighed amount of stock ration. The ether must be evaporated while the mixture is being stirred to facilitate an even distribution of the cholesterol. This procedure is elaborate, and an even distribution is not easily achieved. The food does not entirely lose the smell of ether, which is repulsive to the animals. A day or two of starvation is necessary before the mixture will be eaten by the rabbit and no other food can be given until it is all eaten. Quite frequently, a rabbit who has been eating the food, will suddenly refuse it, requiring a new period of starvation. Such a period may upset the whole experiment by changing the animal's weight and the amount of cholesterol consumed. So, although this method may eliminate many of the fallacies of tube feeding, it has many new disadvantages of its own.

For these reasons, as well as for lack of time and assistance, new ways of administering the cholesterol were tried in the laboratories of the Taunton State Hospital. Feeding animals gelatin capsules (no. 00 of Parke, Davis & Company) containing 0.5 Gm. of cholesterol proved to be a suitable procedure. Each capsule holds 0.5 Gm., and the weight can be easily controlled.

Daily for a week a filled capsule dipped in molasses was placed on the rabbit's tongue. Sometimes the animal would voluntarily swallow the capsule, whereas at other times some force had to be applied. The following week a similarly treated capsule was placed in the feeding cup in the cage before the regular feeding time, and in most instances the animal took it readily. From then on the capsule was taken from the cup or out of the attendant's hand. There were some differences in the response of the animals, so that a certain degree of individualization of feeding was found to be necessary.

Most of the disadvantages of the other methods are avoided by this procedure. It is simpler and takes less time. There is no loss of cholesterol, and an exact and uniform dosage is possible. Diarrhea occurs less frequently than when oil is used as a vehicle for the cholesterol. Fewer animals are required, because control series fed empty capsules are not necessary.

Cholesterol is absorbed regardless of the method used in feeding. With our method the blood cholesterol level of rabbits increases to twice the normal within two to three weeks, or after 7 to 10 Gm. of cholesterol in daily doses of 0.5 Gm. have been administered.

SUMMARY

Cholesteremia may be produced in rabbits by feeding them cholesterol in gelatin capsules.

From the Laboratories of the Taunton State Hospital.

Aided by a grant from the Department of Mental Health of the Commonwealth of Massachusetts, for research in arteriosclerosis.

1. Anitschkow, N., and Chalatow, S.: Centralbl. f. allg. Path. u. path. Anat. **24**:1, 1913. Anitschkow, N.: Beitr. z. path. Anat. u. z. allg. Path. **56**:379, 1913.

2. Weinhouse, S., and Hirsch, E. F.: Arch. Path. **30**:856, 1940.

AUTOMATIC STAINING OF ROUTINE TISSUE SECTIONS WITH HEMATOXYLIN AND EOSIN

MAURICE N. RICHTER, M.D., NEW YORK

It is common practice to facilitate the staining of tissue sections by using racks that permit the transfer of many slides at once. Inasmuch as the whole rack of slides is transferred from one solution to another at the same time, the change can be made automatically by the use of the apparatus employed to transfer tissues for dehydration and embedding.

Various formulas and staining schedules can be used. The method herein described has proved satisfactory for the automatic staining of routine sections of pathologic material with hematoxylin and eosin, and it permits modification to obtain the degree of staining and differentiation desired.

The main value of automatic staining is the uniformity of results independent of the experience of the technician. In addition, the staining requires no attention and can be carried out while the technician is otherwise occupied.

METHOD

Sections of material that has been fixed in Zenker's fluid [1] are mounted on slides by the albumin method. The slides are placed in the paraffin oven (56 C.) for one hour and then, in the metal slide rack, are placed in the first solution on the automatic changing machine.[1a] The timing clock is set and started, and the machine carries the slides through the various solutions and leaves them in xylene, ready to be mounted under cover slips.

To convert the machine from dehydration to staining schedules, a separate timing mechanism is supplied, permitting changes at short intervals. In addition, a carrying rack for slides is needed, and provision for running water in one of the containers. A small motor keeps the slides in constant motion while they are in the solutions.

Inasmuch as the machine has only twelve containers, the schedule must be adapted for use with not more than twelve solutions.

The time schedule is as follows:

1. Xylene, 1,000 cc., containing iodine crystals, 10 Gm.	5 minutes
2. Xylene	5 minutes
3. Absolute alcohol	5 minutes
4. 95 per cent alcohol	5 minutes
5. Mayer's acid hemalum 100 cc. 5 per cent ammonium alum 900 cc.	25 minutes
6. Running water	5 minutes
7. 95 per cent alcohol with eosin	1 minute
8. 95 per cent alcohol	5 minutes
9. Absolute alcohol	1 minute
10. Carbolxylene	1 minute
11. Xylene	1 minute
12. Xylene	1 minute or more.

From the Department of Pathology, New York Post-Graduate Medical School and Hospital, Columbia University.

1. This is Zenker's fluid without the addition of acetic acid or solution of formaldehyde. Either may be added, however.

1a. The equipment used is the Autotechnicon and accessories manufactured by the Technicon Company, New York.

NOTES

The numbers of the following paragraphs refer to the corresponding numbers of the steps in the foregoing staining schedule.

1. Using iodine in the first xylene instead of in alcohol or in water for the removal of mercuric salts makes it unnecessary to use sodium thiosulfate. The iodine is readily soluble in xylene and is sufficiently removed by the second xylene and following alcohols so that no interference with subsequent staining occurs.

If tissue is fixed in solutions not containing mercuric chloride, iodine is unnecessary.

2. The times recommended in the second, third and fourth steps allow for removal of iodine before staining. If iodine is not used, these times can be reduced to one minute each.

4. It is unnecessary to use water before hematoxylin solutions. In the case of hematoxylin solutions containing alcohol the preliminary use of water reduces the alcoholic content of the stain and causes more rapid deterioration.[2] The omission of water before hemalum in this technic causes no difficulty.

5. The use of acid hemalum instead of the more commonly used hematoxylins is suggested because differentiation in acid is unnecessary and the solution is easier to prepare. Lillie's formula [3] is used because of its better keeping qualities:

Hematoxylin	5 Gm.
Sodium iodate ($NaIO_3$)	1 Gm.
Ammonium alum $(AlNH_4(SO_4)_2 + 12H_2O$	50 Gm.
Distilled water	700 cc.
Glycerin	300 cc.
Glacial acetic acid	20 cc.

Dissolve the alum in the water, then the hematoxylin, then the iodate. When the color change has occurred, add the glycerin and the glacial acetic acid. No ripening is necessary.

The use of an alkali can be omitted, as tap water is usually alkaline enough to cause bluing of the hematoxylin.

If slight diffuse staining with hematoxylin is not considered objectionable, the full strength hemalum solution may be used and the time reduced to two minutes. My associates and I prefer, however, the more specific nuclear stain obtained with dilute solutions and longer staining time. With the dilution used, the results are practically the same as with Harris' or similar hematoxylins.

6. The time in running water should be sufficient to give the desired blue color to nuclei and may vary with different water supplies.

7. The eosin can be used in either alcoholic or aqueous solution and according to any desired formula.

2. Von Glahn, W. C.: Personal communication to the author.

3. Lillie, R. D.: Stain Technol. **17**:89, 1942.

We prefer an alcoholic solution of an acid precipitate recommended by Fischer[4] and prepared as follows: Take distilled water, 1,000 cc., and eosin Y, soluble in water and in alcohol, 10 cc. Add concentrated hydrochloric acid until precipitation is complete. Filter and wash the precipitate thoroughly with distilled water until the filtrate is no longer acid to litmus. Dry the precipitate at 37 C. and prepare a saturated stock solution in 95 per cent alcohol, leaving undissolved precipitate at the bottom. For use, take 250 cc. of the stock solution and 750 cc. of 95 per cent alcohol.

4. Fischer, cited by Hodenpyl, E.: M. Rec. **53**:351, 1898.

The time in eosin can be varied according to the intensity of stain desired.

8. This time can be reduced, but at a slight sacrifice of differentiation.

12. The slides remain in this solution until mounted.

All containers should be filled (1 liter) regardless of the number of slides stained. In changing solutions, nos. 1, 2, 4, 5, 8 and 11 have been discarded weekly. No. 3 has been moved to 4, 9 to 8, and 12 to 11. The hemalum can be used longer. Its staining properties are not exhausted but deteriorate after 300 to 500 slides have passed through the solution. Inasmuch as only 100 cc. of the stock solution is used in the working solution, we have not considered it necessary to extend its use.

Books Received

HOMICIDE INVESTIGATION. PRACTICAL INFORMATION FOR CORONERS, POLICE OFFICERS AND OTHER INVESTIGATORS. By LeMoyne Snyder, medicolegal director, Michigan State Police, member of the American Medical Association and member of the American Bar Association. With chapters by Captain Harold Mulbar, chief of the Identification Bureau of the Michigan State Police; Charles M. Wilson, director, Chicago Police Scientific Crime Detection Laboratory, and C. W. Muehlberger, director, Michigan Crime Detection Laboratory. Pp. 287, with 116 illustrations. Price $5. Springfield, Ill., and Baltimore: Charles C Thomas, Publisher, 1944.

In recent times significant advances have been made in the scope and the technic of investigations of homicides. A most important advance is the increasing realization that the outcome of this work may depend mainly on its being done competently before any changes whatever are made at the scene of death. How such an investigation should be conducted is described in detail in the book by Snyder and his associates. The book is clearly written and well illustrated. It will be of great help to officials whose duty it is to determine the causes of deaths which may be due to violence. The book will be of interest and value as well for the pathologist or the physician who may be required to make necropsies in the course of such investigations.

THE AMERICAN ILLUSTRATED MEDICAL DICTIONARY. By W. A. Newland Dorland, M.D., Lieut.-Colonel, Medical Reserve Corps, United States Army; member of the Committee on Nomenclature and Classification of Diseases of the American Medical Association; editor of the "American Pocket Medical Dictionary." With the collaboration of E. C. L. Miller, M.D., Medical College of Virginia. Twentieth edition, revised. Pp. 1,668, with 885 illustrations. Price: plain, $7; thumb-indexed, $7.50. Philadelphia and London: W. B. Saunders Company, 1944.

The revision has been extensive and thorough, with "additions and alterations on every page." Hundreds of new words have been defined. The vocabulary of war medicine has received particular attention. The nomenclature and definitions of diseases and operations now conform to the "Standard Nomenclature of Diseases and Operations" published by the American Medical Association under the editorship of Dr. Edwin O. Jordan. Among the agencies aiding in the revision were the editorial departments of the American Medical Association and the Mayo Clinic, and the staffs of the libraries of the New York Academy of Medicine and the College of Physicians of Philadelphia. The dictionary has a notable record. The first edition was published in 1900. The twentieth edition will maintain fully the practical usefulness of the book.

Forensic Medicine

SCIENTIFIC EVIDENCE IN CASES OF INJURY BY GUNFIRE

ALAN R. MORITZ, M.D., AND FRANK R. DUTRA, M.D.
BOSTON

In no type of death by violence is a painstaking search for scientific evidence likely to be as richly rewarded from a legal standpoint as in that of fatal injury by gunfire. The finding of the body of a person dead of an unwitnessed or unreliably witnessed shooting invariably raises a host of pressing questions. It is essential that the truth be learned as quickly as possible. Did death result from homicide, was it suicide, or could it have been an accident? From what kind of weapon was the fatal shot fired and from what distance and from what direction? What kind of ammunition was used? What can be learned regarding the individual characteristics of the gun that was used? If multiple wounds are present, in what sequence were they incurred, and how many different weapons or different bullets were responsible for their production? How long did the victim survive the injury, and how soon was he incapacitated?

Almost invariably the answers to many of these questions are to be found in, on or near the body. Because of the ease with which critical objective evidence may be destroyed or lost, no police officer, medical examiner or coroner's physician should undertake to investigate the scene of a shooting or the body of a person dead of gunfire unless he knows what to look for and how to preserve its evidential value.

The purpose of this paper is to call attention to the principal sources of objective evidence in cases of gunshot injury and to indicate the potential value of such evidence in the administration of justice. To appreciate the significance of objective evidence derived from victims of gunfire it is necessary that the reader be familiar with certain of the basic characteristics of firearms and ammunition.

FIREARMS

Rifled and Smooth Bore.—Firearms may be divided into two main classes according to whether the inner surface of the barrel is rifled or smooth. In the former, spiral ridges engage the surface of the bullet as it passes through the barrel and give it a centrifugal rotation that increases the accuracy of its flight. Smooth bore weapons do not contain such ridges and depend on the dispersion of multiple simultaneously discharged projectiles for their effectiveness rather than on the accuracy of an individual bullet. The barrels of rifles and pistols are rifled, whereas those of shotguns are smooth.

Single Shot and Repeating Guns.—Both rifled and smooth bore weapons are in turn divided into two main subgroups according to whether they can be fired repeatedly or only once without reloading. A single shot weapon is one that has no magazine or other device for holding extra cartridges. It must be reloaded after each firing, and the new cartridge must be placed in the gun by hand. A variant of the single shot type of firearm is one with a double barrel. Such a weapon is in reality two single shotguns in one, and although it can be fired twice without reloading, each barrel must be loaded manually before it can be fired again. The double barrel shotgun is the only modern example of such a weapon.

There are two principal types of repeating rifles and shotguns. In each the gun is equipped with a magazine for holding extra cartridges. The type of gun conventionally described as a "repeater" is one having a manually operated device in the form of a sleeve, a bolt or a lever for ejecting the empty case of the fired cartridge and reloading the barrel with a fresh one. The type of gun conventionally described as automatic is in reality semiautomatic. In weapons of this type the force of the backward thrust of the exploding cartridge is used to eject the empty shell case and to reload the barrel. Semiautomatic repeaters differ from those that are truly automatic in that the trigger of the former must be pulled each time the gun is fired. Fully automatic weapons, such as the machine gun, continue to fire as long as pressure is maintained on the trigger.

One form of pistol has a revolving type of repeating mechanism. The extra cartridges are kept in a revolving cylinder, which is constructed in such a manner that each of its bullet-containing compartments becomes an integral part of the barrel when rotated into the firing position. There are no truly automatic pistols. Semiautomatic pistols, commonly designated as auto-

From the Department of Legal Medicine, Harvard Medical School.

matic, operate on the same principle as do semiautomatic rifles and shotguns.

Caliber.—The caliber or internal diameter of the barrel of a rifled weapon is usually expressed in hundredths of an inch. The calibers of the most commonly used firearms in the United States are .22, .25, .32, .38 and .45.

The calibers of the more commonly used shotguns are ordinarily designated by gage numbers. The gage number is actually a rough reciprocal of the weight of a lead ball having a diameter equal to that of the barrel. The internal diameters of the more commonly used shotguns expressed in hundredths of an inch together with their gage equivalents are as follows: .775 (10 gage), .729 (12 gage), .662 (16 gage), .615 (20 gage). One small caliber shotgun having an internal barrel diameter of .410 inch is not ordinarily designated by its gage equivalent.

AMMUNITION

Although there are major differences between the ammunition for rifled and that for smooth bore weapons, as well as minor differences within each class of ammunition, cartridges for modern weapons have certain basic similarities. A cartridge consists of one or more projectiles and a powder charge all contained in a case or shell which keeps the charge and the projectiles together and protects them against loss or deterioration. In addition to these three basic components most cartridges also have a primer or cap. The cap is a soft metal cup containing a highly explosive mixture and is incorporated into the center of the base of the shell. Its construction is such that when it is struck by the firing pin or hammer of the gun its contents are exploded. This explosion of the cap ignites the main powder charge.

The rapidly expanding gases which emanate from the burning powder propel the projectile through the barrel and emerge from the muzzle. Some smoke may escape from the breech in the case of revolvers in which the fit between cylinder and barrel is loose.

Powder Charge.—Different kinds of explosive mixtures are used in different kinds of ammunition. Some contain black powder, some smokeless and some a combination of black and smokeless called semismokeless. Because smokeless is a better propellant than black powder, it is used more frequently. Ammunition for revolvers, shotguns and small caliber rifles is occasionally charged with black or semismokeless powder. Ammunition for semiautomatic pistols and high velocity rifles is almost invariably charged with smokeless powder. Although any one of the three types of powder may be used in ammunition for shotguns, the smokeless is probably most commonly encountered.

Black powder is a mixture of potassium nitrate, sulfur and charcoal. Smokeless powder consists of cellulose nitrate alone or in combination with nitroglycerin (glyceryl trinitrate). With each type of powder, both burned and unburned components of the charge may be discharged from the muzzle and may be recognized on the target by their distinctive physical and/or chemical characteristics.

Primers.—Since the combustion products of the primer mixture are also discharged from the muzzle, a knowledge of their chemical composition is desirable. Formerly mercury fulminate, stibnite, potassium chloride and powdered glass were used almost exclusively. Recently this mixture has been supplanted in some ammunition by a combination of lead azide, lead styphnate and barium nitrate. Stibnite is still used occasionally, and to some primers zirconium has been added.

Projectiles.—Projectiles vary as to shape, size, composition and number per cartridge according to the type of gun and the use for which they are intended. Cartridges for rifled firearms contain a single projectile or bullet, which is characteristically elongated, with a rounded or tapered forward end. The diameter of the bullet corresponds to the bore of the weapon for which it is intended. Bullets for revolvers and low velocity rifles are ordinarily comprised of lead which may or may not be alloyed with antimony or tin or both. Some lead bullets have a thin plating of cadmium, copper, tin or zinc. Bullets for most rifles greater than .22 caliber and for semiautomatic pistols are usually jacketed. The core of such a bullet is lead, and the jacket is usually comprised of cupronickel, steel and cupronickel, or brass and cupronickel. Lead bullets are frequently lubricated with petrolatum or graphite.

Cartridges for shotguns are usually loaded with multiple lead spheres. These fall into three main groups according to the size of the individual pellets. Birdshot starts with spheres so small that they are characterized as dust (.04 inch in diameter) and the size of the spheres ranges upward to a diameter of .23 inch. The diameter of buckshot varies from .24 to .36 inch. The diameter of lead balls varies from .51 to .71 inch. A type of shotgun cartridge intended for deer hunting contains a single large spherical or elongated projectile. Some ammunition of this type shows longitudinal grooves cut in the projectiles in such a manner that the friction between them and the barrel will tend to give them a rotary motion.

Shell Cases.—The shell case of a cartridge for use in a rifled weapon is metal and has either a projecting rim or a groove at the margin of the base to facilitate its extraction from the barrel. Cartridges for single shot guns, for manually operated repeaters and for revolvers have a projecting rim, and cartridges for semiautomatic rifled weapons have a circumferential groove. The name of the firm that manufactured the ammunition and the caliber of the gun for which it is intended are sometimes imprinted on the base of the case. Cases for use in shotguns are usually made partly of metal and partly of cardboard.

Wads.—In addition to the components already described, cartridges for shotguns contain disks of cardboard and felt. One disk of cardboard closes the forward end of the shell case and keeps the shot from spilling out. Additional disks of cardboard and felt separate the shot from the powder charge. These disks are discharged from the muzzle when the gun is fired.

Types and Sources of Evidence of Value in Cases of Death by Gunfire

	Where Information May Be Obtained										
	Empty Shell Case				Spent Bullet				Powder Residues		
Information Desired	Location of	Number of	Marks on	Contents of	Location of	Number of	Marks on	Traces of	Pattern of	Composition of	Wounds
Individual peculiarities of gun	..	..	+	..	..	..	+	..	..	..	..
Make and model of gun	..	..	+	..	..	..	+	..	..	..	..
Kind of ammunition	..	..	+	..	..	..	..	+	..	+	+
Range of fire	+	..	..	..	..	..	..	..	+	..	+
Direction of fire	+	..	..	..	+	..	..	..	+	..	+
Number of shots fired *	..	+	..	..	..	+	..	+	+	..	+
Time shooting occurred	..	..	..	+	..	..	..	..	..	..	+
Time elapsed before injury became disabling or fatal	..	..	..	..	..	..	..	..	..	..	+

* Some types of evidence (shell cases or spent bullets) would indicate that a corresponding number of shots had been fired, whereas other evidence (number of original entrance wounds in target) indicates number of projectiles which struck target.

SOURCES OF EVIDENCE

The accompanying table shows the kind of information that is usually desired and the principal sources from which it may be obtained.

Empty Cartridge Cases.—(*a*) Location: Obviously the finding of an empty cartridge case in the vicinity of a person dead of gunfire does not necessarily indicate that it is the case from which the fatal shot was fired. However, it may become an exhibit of great importance. If the gun that was used was a single shot, a manually operated repeater or a revolver, an empty cartridge case or cases may or may not be found at the place. If, however, a semiautomatic weapon was used the empty cases will usually be found near the place where the shooting occurred and within a few feet of the place where the gun was operated.

The distance between the place where the gun was fired and the place where the body was found may be such as to exclude the possibility of suicide. The location of the empty shell cases may indicate that testimony purporting to show that the gun was fired in self defense is probably false.

(*b*) Number of shell cases: The number of empty shell cases found may fail to agree with the number of shots said to have been fired and may indicate that what originally appeared to be an accident is actually a murder.

(*c*) Marks: An examination of a recovered shell case will determine the kind of ammunition that was used and possibly the kind and the caliber of gun from which it was fired. Empty shell cases often bear imprints or abrasions of such a highly individual character as to constitute a personal signature of the weapon from which they were fired.

Such individual characteristics are derived from several sources. The most important of these is the breech face. When a gun is fired the base of the cartridge is expanded backward against the breech face with great force. Whatever irregularities there are on this surface are likely to be imprinted in the base of the cartridge and particularly in the ductile metal that comprises the primer cap. Individual peculiarities in breech faces may consist of the file or the machine marks that were left when the surface of the breech face received its last finishing, or they may be caused by wear and tear.

Other individual marks on the base of the shell case may be derived from the firing pin or hammer. Irregularities of the surface or of the alinement of either, whether they are original or acquired, may leave distinctive impressions on the primer cap. Peculiarities in the extractor or the ejector mechanism may also lead

to characteristic abrasions on the edge of the base of the shell case. If the individual characteristics of a shell case from the scene of the crime are identical with those of a shell case known to have been fired from a certain gun it can be assumed that the case from the scene of the crime was fired in that gun.

(*d*) Contents: In favorable circumstances chemical analysis of the combustion residues remaining in the empty cartridge case may provide information regarding the time that elapsed between firing and examination. Certain chemical changes in the burned powder that has been left in the case may occur as a result of exposure to air. These changes are usually completed within a few hours, and if they have not yet reached completion when the shell is examined, it may be possible to state that the cartridge was recently fired.

The importance of determining the location of a bullet that has passed through the body may be illustrated by a case in which homicide was suspected by reason of the fact that the victim was shot from in front and from a distance that was too great for suicide. It was known that there had been an altercation between the victim and the suspect. The finding of the spent bullet in the ceiling directly above the victim's body lent support to the allegation that the injury was accidental and occurred when a revolver which the victim had carried in his inside coat pocket fell to the floor and discharged as he leaned forward to pick up something which he had dropped. If the bullet had not been located, it is reasonable to assume that the suspect would have been charged with murder.

(*b*) Number of bullets: Reliable information concerning the total number of shots fired is

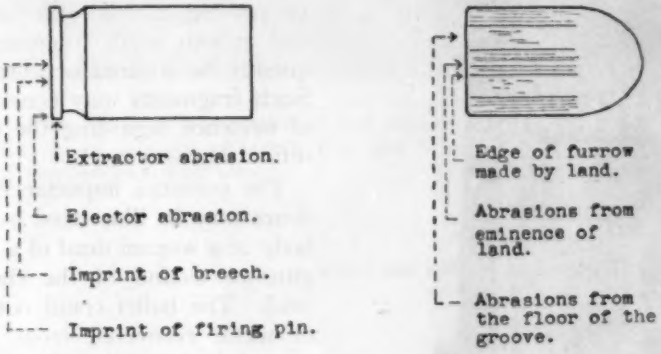

Fig. 1.—Diagrammatic representation of empty cartridge and fired bullet showing the principal sources of imprints and abrasions likely to be useful in establishing the identity of the gun from which the bullet came.

Thus, to summarize the information that may be gained from an examination of empty cartridge cases, it may be possible to establish: (1) the place (range and direction) of gunfire; (2) the number of shots fired; (3) the kind of gun that was used; (4) the individual characteristics of the gun; (5) the kind of ammunition that was used; (6) the length of time that elapsed between the firing of the gun and the finding of the empty shell.

Bullets.—In no instance of fatal gunshot injury can the search for evidence be regarded as complete until all the bullets that were fired, including those that missed the target, have either been found or the possibility of finding them exhausted.

(*a*) Location: It is obvious that information regarding the direction of fire will often be disclosed by the projection of a line between the place where the bullet first struck the target and the place where it finally came to rest. It is essential, of course, that proper allowance be made for deflection and secondary migration.

always desirable and can frequently be obtained by a careful examination of the scene of a shooting. The finding that a single shot was fired sometimes points to a suicide or an accident, whereas the finding that multiple shots were fired may justify a suspicion of murder. If, for example, in a case of fatal gunshot injury it is determined that only one shot was fired, it might be concluded on the basis of collateral evidence that the manner of death was accidental. However, the finding of evidence that several shots had been fired and that only one had hit the target might easily point to murder.

A case in point is that of a woman who was found dead of a through and through gunshot injury of the head. Bilateral temporal wounds were regarded as the entrance and exit holes of a single bullet. In view of the fact that there was blackening of the wound of the right temple, that a gun was found beside the body and that the victim was known to have been predisposed to suicide, it was assumed that the fatal injury had been self inflicted. However, at autopsy a

bullet was found in the brain, indicating that more than one shot had been fired. The bullet in the brain had produced the entrance wound in the right temple. A search was then made for the bullet that had produced the entrance wound in the left temple. This bullet was eventually found in a window sill in a place that indicated that it had been fired from the victim's left side, had passed through the head, and emerged through the hole that the first bullet had made in the right temple. The two bullets in this case and their location provided evidence that the manner of the woman's death was homicide and not suicide.

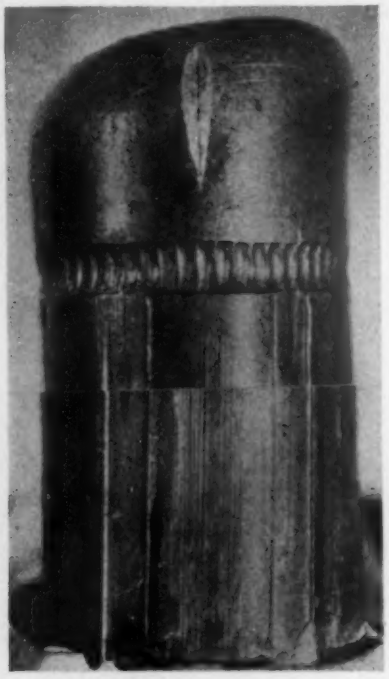

Fig. 2.—Composite photograph: The lower half is the test bullet and the upper half is the bullet under investigation. The number, the width and the pitch of the lands indicate that they came from the same make and model of gun and the similarity of secondary abrasions produced by irregularities in the lands and the grooves indicate that they came from the same gun. (Captain Charles J. Van Amburg supplied this illustration.)

(*c*) Markings on bullets: The evidentiary value of the marks imparted to a bullet from the barrel through which it has passed are well known. Differences in the number, the direction, the pitch and the width of the grooves and ridges that have been cut in the inner surface of the barrel make it possible to identify the make and model of a firearm by the marks that have been left on a bullet.

In addition to the marks that indicate the make and model of the gun, a bullet will frequently bear marks of a highly individual character. As a result of wear and tear, barrels acquire certain irregularities that will not be present in an identical fashion in any other firearm. If these individual irregularities are sufficiently prominent, they will produce corresponding marks on bullets. It is apparent, then, that when it can be demonstrated that a bullet found in a body and a test bullet fired in the laboratory have identical marks, and that the marks could have been produced only by the irregularities of a single barrel, the gun from which the test bullet was fired is the same one that was responsible for the bullet in the body.

(*d*) Traces of bullets: Even though only few traces of a bullet can be found, as may be the case when the bullet has passed through the body and come to rest in some unknown location, appropriate chemical or spectroscopic examination may provide important information regarding the kind of ammunition that was used. In passing through the skin both at entrance and at exit small fragments of metal will frequently be sheared or abraded from the bullet. Such fragments may constitute the only source of evidence regarding the nature of the ammunition that was used.

The potential importance of this kind of evidence may be illustrated by a case in which the body of a woman dead of a through and through gunshot wound of the chest was found in a field. The bullet could not be located. Traces of metal recovered from the margins of the wound disclosed that this metal was lead heavily alloyed with tin. It was shown that the only ammunition possessed by a suspect who had been charged with the crime was of an entirely different composition. Not only was the composition of the bullet that killed the woman different from that of the ammunition in possession of the suspect but it was also different from that of any standard brand of ammunition. It was subsequently concluded that the bullet that killed the woman was one that had been cast from soldering metal by a person who manufactured his own ammunition.

In recapitulation the following facts deserve emphasis. So far as possible all bullets should be recovered and the location of each should be accurately recorded. Even when it appears that the entire truth regarding the circumstances and the manner of death is already known, an attempt should be made to confirm the facts by objective evidence. In every case of fatal gunshot injury there should be sufficient study of the physical and chemical characteristics of bullets or traces of bullets to determine whether the bullet was, could have been, or could not have been fired from the suspect gun. Great

care should be exercised in the marking of bullets for identification purposes. If a bullet is to be marked for identification, a fine steel point should be used, and the mark should be placed in the center of the base.

Powder Residues.—Attention has already been called to the fact that different kinds of powder have different chemical and physical properties and that the traces of powder that emerge from the muzzle of a gun with the bullet may be deposited on the target if the distance between target and gun is not too great.

(*a*) Pattern: The blast of particulate matter and gas that emerges from the muzzle tends to assume the shape of a cone, and the greater the distance between target and muzzle the greater will be the area of fouling on the surface of the target. The heaviest elements of the blast are carried the farthest. The wads of a shotgun shell may be carried ten yards or more. Droplets of mercury from the primer or bits of metal that have been sheared or melted from the bullet may travel 3 or 4 feet (91 or 121 cm.) in a relatively straight line. Ordinarily it is difficult to see much fouling with the naked eye if the distance between target and muzzle was more than 24 inches (61 cm.). No exact estimate of distance can be made unless test targets

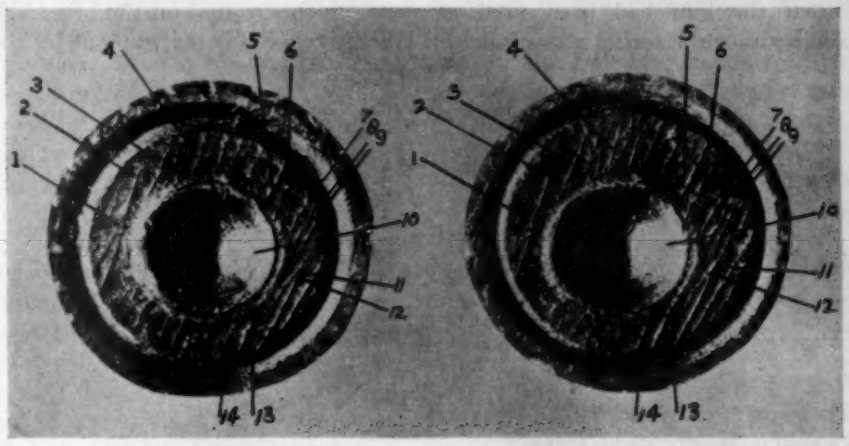

Fig. 3.—Photograph of the bases of the primers of two empty cartridge cases which were fired from the same weapon. Similarities in the imprints of breech face and firing pin are indicated by numbers. (Captain Charles J. Van Amburg supplied this illustration.)

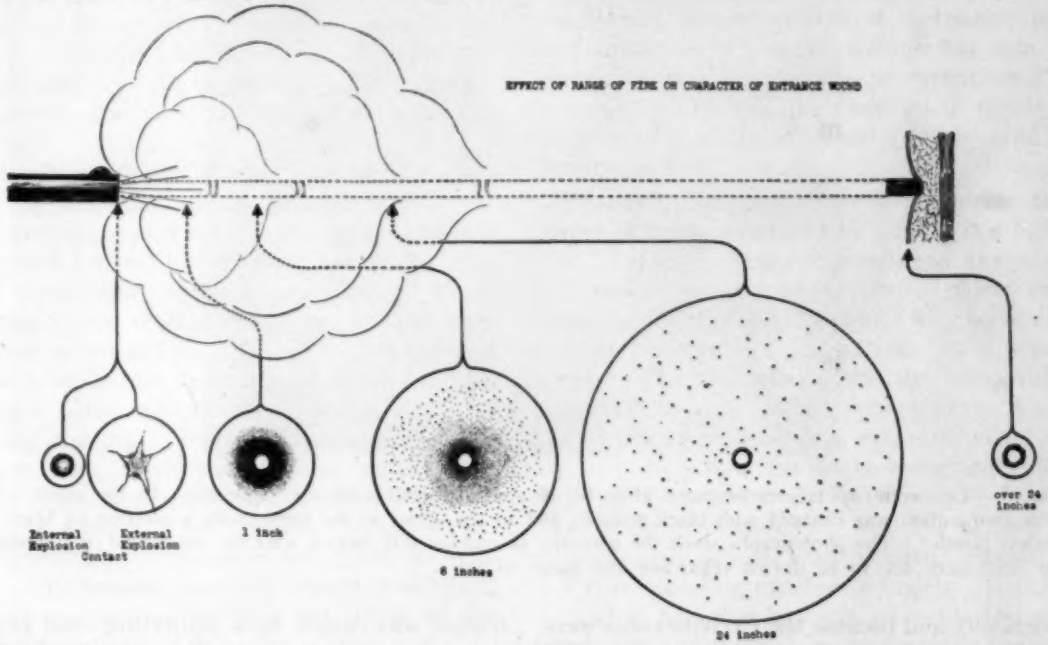

Fig. 4.—Diagrammatic illustration of the muzzle discharge of a firearm and the effect of its various components on targets at different ranges of fire.

are prepared with the gun and the ammunition that were used and are compared with the target that is being investigated.

The importance of preparing test targets with the gun and the ammunition that were used in the shooting being investigated is illustrated by the case of a hunter who was found dead in the woods with a shotgun wound of the chest. It was obvious that the gun had been fired at close range, and because the victim was in financial difficulty and because the circumstances were at first considered not to be consistent with accident, the manner of death was certified as suicide. Several insurance policies were invalidated by this finding. A reinvestigation of the case threw doubt on the official verdict because of evidence that the range of fire was greater than is usually the case in suicide. A series of test shots were made with the same gun and ammunition, cloth identical with that of the hunting jacket of the man who was shot being used for the target. Repeated experiments showed that the pattern of the powder residue around the hole in the hunting jacket was characteristic of a range of fire of not less than 12 inches (30.5 cm.) and not more than 18 inches (45.5 cm.). A reexamination of all of the evidence indicated that the man had probably rested the barrel of his gun on a fallen log with the butt on the ground on the other side of the log. When he picked the gun up by the barrel, the trigger was caught by a projecting dead branch and the gun discharged. This reconstruction of the circumstances was in accord with the objective evidence bearing on the range of fire, and the insurance claims were paid.

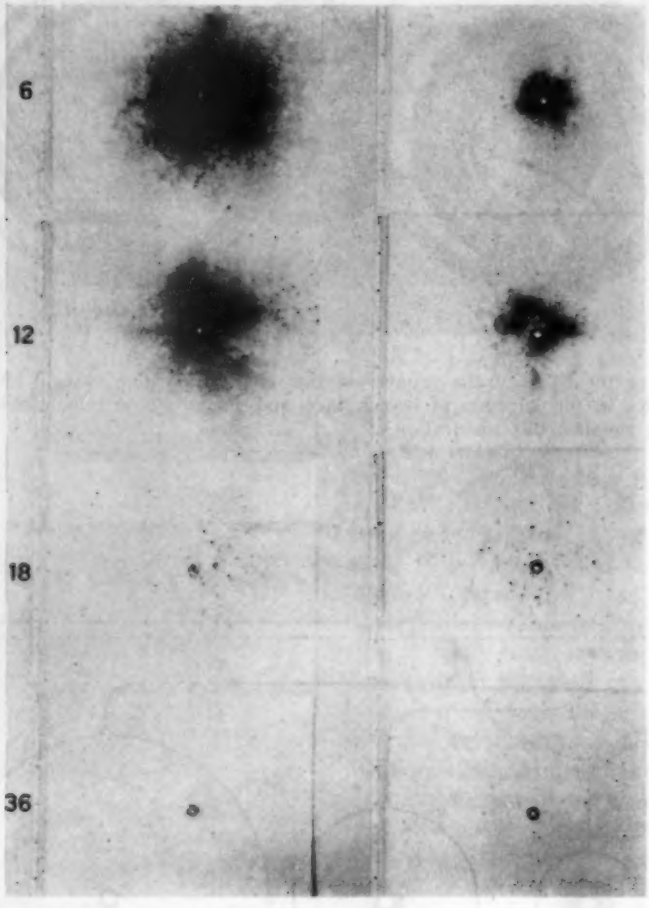

Fig. 5.—Two series of targets prepared at 6, 12, 18 and 36 inches with the same gun. In the series at the left the ammunition was charged with black powder, and in the series at the right, with a mixture of black and smokeless powder. The photographs show the necessity of making test targets with the same kind of ammunition if any inferences are to be drawn regarding the range of fire.

As already indicated, when the same gun and ammunition are used, the size of the powder pattern tends to increase with the range of fire. However, this is not invariably the case. If the muzzle of the gun is fired in tight contact with the target, the gases and residue of the explo-

sion may be so completely blown into the wound as to be invisible by external examination. If the contact between muzzle and target is loose, the combustion products may be blown radially over the surface in such a manner as to produce a wider area of soiling than would occur if the muzzle had been several inches away. When the distance between muzzle and target is less than an inch, the pattern of the soiling is likely to be characterized by radiating bands of intense discoloration disposed around the bullet hole like the spokes of a wheel.

Generally speaking, the pattern of a powder residue is likely to be more homogeneous and to show less stippling when the range of fire is between 1 and 6 inches (2.5 and 15 cm.) than when it is over 6 inches. It should be remembered that black powder on combustion produces more residue and is more likely to produce prominent stippling and punctate burning on the surface of the target than smokeless powder.

The position of the bullet hole in relation to the powder pattern may provide information as to the direction of fire. Other things being equal, the presence of the bullet hole in the center of the powder pattern indicates that the barrel was directed approximately at right angles to the surface of the target at the moment of firing. When the long axis of the barrel is not at right angles to the target, the bullet hole will be eccentric and close to the near edge of the pattern.

(*b*) Composition: Recognition of the physical characteristics of the powder residue so often provides useful information that every target, whether it is skin or cloth, should be routinely examined with a magnifying glass. Burning of the surface of the target invariably means close range fire. The amount of burning that will occur and the range at which burning may take place vary according to the ammunition and the gun. Black powder causes more burning than smokeless. Cloth burns more readily than skin, and hair is more likely to show the effects of heat than is the epidermis. The presence of microscopic bubbles in the hair shafts may provide the only objective evidence that the muzzle was within a few inches of the target when fired.

In residues produced by black powder relatively large amorphous granules are likely to be identified. Residues produced by smokeless powder frequently contain unburned elements of highly characteristic appearance. Smokeless powder is usually comprised of uniform particles of geometric contour. The contours of such unburned particles may be so characteristic that the firearms expert may be able to identify the type of ammunition that was used by the size and the shape of single unburned particles of the powder.

Not infrequently the target is such that even though powder residues are present they cannot be recognized by ordinary inspection. This is particularly true when the target is a piece of dark cloth. Several physical methods are available to the investigator in such circumstances. One is the employment of infra-red photography. Powder residues on cloth that are not visible to the naked eye frequently can be identified in an infra-red photograph. Invisible residues can also be demonstrated by soft roentgen rays (rays of long wavelength and little penetrative power).

Appropriate chemical examination of a target may disclose an unsuspected powder residue. Even though the powder residue has been recognized visually, chemical tests to determine its exact composition may disclose the kind of ammunition that was used. A comprehensive review of the various chemical procedures that may be used to identify the composition and the distribution of powder residues has been published by Walker.[1]

Thus, to summarize the information to be gained from the recognition of the nature and the distribution of powder residues on skin or clothing, it may be possible to determine (1) the range of fire, (2) the direction of fire, (3) the number of shots fired and (4) the kind of ammunition used.

Wounds.—The most impressive evidence that a given hole was produced by gunfire is a bullet in it. Under appropriate conditions any small hard missile or any rigid pointed object may produce a defect in the skin which may be similar in many respects to a bullet hole. It is a fact, however, that the velocity and the composition of a bullet usually impart certain physical and chemical characteristics to a wound whereby the manner of its production can be recognized if it is subjected to competent investigation.

(*a*) Number of wounds: Multiple wounds do not necessarily indicate that the victim was struck by a corresponding number of bullets. It is not unusual to find that a single projectile has produced several different sets of entrance and exit wounds. In a case in point seen by one of us (A. R. M.) the victim of a shooting affray was leaning forward and running toward his assailant when the fatal shot was fired. The man with the gun was standing on an eminence, and the bullet first struck the top of the victim's shoulder, emerged from the front of his chest, grazed the

1. Walker, J. T.: J. Crim. Law & Criminol. **31**: 497, 1940.

abdomen, passed through the inner aspect of the left thigh, and emerged from the thigh to enter the calf of the leg, where it shattered the tibia and produced four exit wounds, at least one of which was caused by a fragment of bone rather than by the bullet. Thus one bullet was responsible for ten separate defects in the skin.

(*b*) Location of wounds: The fact that on external examination no wound is visible does not always exclude the possibility of injury by gunfire. One of us (A. R. M.) has encountered 2 instances of murder by gunfire in each of which the skin of the victim was intact. In one a screaming woman was shot in the mouth. Her lips, tongue and soft palate escaped injury, and the entrance wound occurred at the back of the pharynx. The shooting was unwitnessed, and the bleeding from the mouth was at first ascribed to natural causes. In another instance at the conclusion of a sexual assault the muzzle of a revolver was introduced into the vagina and fired.

(*c*) Appearance of wounds: When a bullet strikes skin or cloth, it indents the surface so that the hole is made through a stretched target. After such a target has been penetrated, it usually contracts and the hole becomes smaller. Thus an entrance wound is frequently smaller than the bullet that produced it.

During the time that the skin or the cloth is being stretched over the nose of the bullet, it is bruised, abraded and soiled over an area that is considerably larger than the hole itself. It is usually possible to identify the direction in which the bullet was traveling by an examination of the margins of the hole.

In cloth the side of the fabric which shows soiling at the margin of the hole will be the surface that was first struck by the bullet. In skin the marginal soiling, contusion and abrasion will be external and readily visible at the site of the entrance wound, while it will be internal and more difficult to recognize at the site of an exit wound. An entrance wound in cloth is shown in figure 6. The contrast between the entrance and exit holes produced by the same bullet in skin is shown in figure 7.

The amount of marginal soiling around an entrance wound varies according to the kind of projectile that produced it. Lubricated lead bullets cause more soiling than unlubricated jacketed projectiles. If the bullet has already passed through several layers of cloth, there will be less fouling of the edges of the wound in the skin than when an unclothed part of the body has been struck.

Bullets traveling at high velocity and bullets that are tumbling as a result of having struck something else before hitting the skin may produce extremely large and irregular entrance wounds. Another circumstance in which a large entrance wound may be produced is that of the muzzle of the gun being pressed so tightly against the skin that the entire blast of expanding gas is carried into the tissues. It is then not unusual to find an enormous explosive type of injury.

Not only are exit wounds characteristically larger than the corresponding entrance wounds but it is not uncommon to find multiple exits associated with a single entrance. Multiple exits may result from the fragmentation of a bullet within the body or from fragmentation of bone.

In some instances it is possible to reconstruct the direction of fire by projecting a line between the entrance and the exit wound. No significance should be attached to this line, however, unless the full track of the wound through the body has been explored in order to determine the extent to which the original direction may have been altered by deflection.

Even though the bullet has passed entirely through the body and even though the surface of the body has been destroyed by putrefaction or fire, it is frequently possible to determine both the direction in which the bullet was traveling and its chemical composition. When a bullet strikes a bone pieces of the bone are displaced in the direction of the bullet's flight. Not only does the manner in which these fragments are dispersed indicate the direction of flight, but information in this regard may also be obtained from the contour of the bony defect. When a bullet passes through a bone it will usually be found that the hole on the side of entrance is considerably smaller than the hole on the side of exit. Information concerning the composition of the bullet can be derived from chemical or spectroscopic analysis of the fragments of metal that are almost invariably found on the broken edges of the bone.

(*d*) Immediate incapacity due to gunshot injury: Occasionally, as is the case when a bullet has destroyed the brain stem or the spinal cord, it can be asserted with confidence that the victim could not have moved himself from the place where the injury was incurred. In other circumstances great caution must be exercised in expressing an opinion as to the extent to which the victim may have been capable of locomotion. It is by no means uncommon for a through and through wound of the brain to be survived without loss of consciousness or of ability to move about. Persons with fatal bullet wounds of the

heart or the aorta are sometimes capable of performing astonishing physical feats before collapsing. Multiple wounds, any one of which might seemingly have been instantly fatal, are sometimes self inflicted.

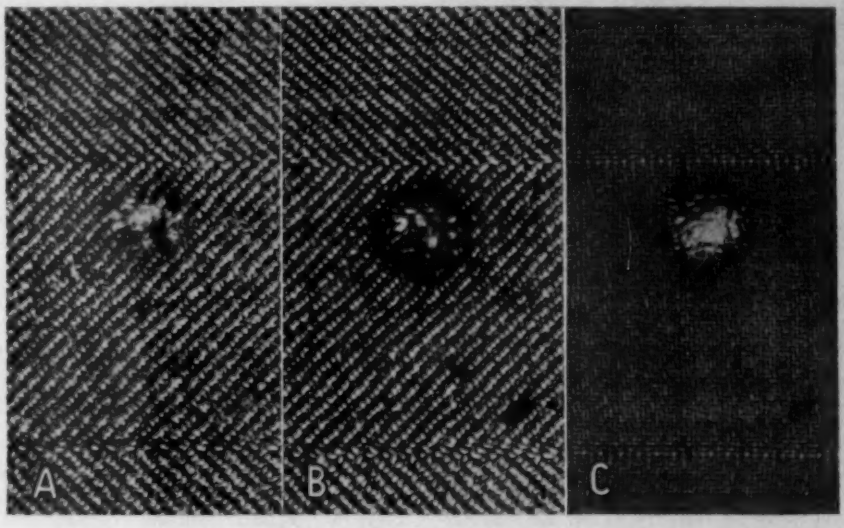

Fig. 6.—Three photographs of the same entrance hole of a bullet in a piece of clothing. *A* was photographed on orthochromatic film and shows the hole and the surrounding cloth as it appeared to the naked eye. *B* shows its appearance when photographed on infra-red film. The contact ring around the hole and soiling of the surrounding fabric by combustion residue can now be seen. *C* is a print from a roentgenogram made with roentgen rays of long wavelength and little penetrative power. It shows the distribution of metallic material in the edges of the hole and in the surrounding fabric. (Dr. Joseph T. Walker supplied this illustration.)

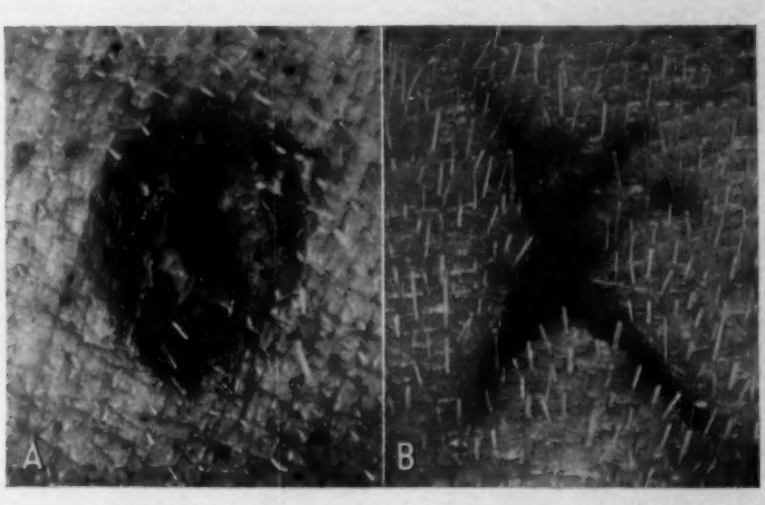

Fig. 7.—Photographs of the entrance (*A*) and exit (*B*) wounds in skin produced by the passage of the same .32 caliber lead bullet through the body. The diameter of the bullet was approximately the same as the diameter of the circular area of abrasion and blackening that surrounds the entrance hole. That the range of fire was close is indicated by the presence of powder grains embedded in the epidermis in the region of the wound. The exit wound is stellate and larger than the bullet and does not show the marginal abrasion and soiling that were present around the entrance wound.

CONCLUSIONS

In no instance of fatal gunshot injury should the circumstances of the shooting or the identity of the firearm that was used be regarded as established until due consideration has been given to all available objective evidence. The principal sources of such evidence are the wounds, the spent bullets, the empty shell cases and the powder residues. Organized society can ill afford to take the risk of allowing a criminal to escape apprehension or an innocent person to be prosecuted because of the failure of the police or the medical investigator to acquire all of the information from these sources that may be relevant to the case.

Notes and News

Appointments.—The Ortho Research Foundation announces that Philip Levine, formerly of Newark Beth Israel Hospital, has joined the research staff as director of the biological division.

Douglas H. Sprunt, since 1932 associate professor of pathology at Duke University School of Medicine, Durham, N. C., has been appointed chief of the division of pathology at the University of Tennessee College of Medicine, Memphis, to succeed Harry C. Schmeisser, who has held the position since 1921 and who has resigned because of ill health. Dr. Schmeisser will continue as professor of pathology.

Granville A. Bennett, head of the department of pathology and bacteriology of Tulane University School of Medicine, New Orleans, has been elected a member of the editorial board of the ARCHIVES OF PATHOLOGY, to succeed the late James Ewing.

Retirement.—Maud Slye, associate professor of pathology at the University of Chicago, will retire from the university July 1. In 1914 she received the gold medal of the American Medical Association for her scientific exhibit on the transmission of hereditary cancer and other diseases in mice; in 1915, the Ricketts Prize of the University of Chicago, and in 1922, the gold medal of the Radiological Society of North America.

Awards.—Ernest W. Goodpasture, professor of pathology and associate dean of the school of medicine of Vanderbilt University, was presented, on May 9, at the fifty-eighth annual meeting of the Association of American Physicians, with the George M. Kober Medal in recognition of his work on viruses. The award is made annually to a member of the association "for outstanding contributions to the progress and achievements of preventive medicine."

Elmer V. McCollum, professor of biochemistry of the Johns Hopkins University School of Hygiene and Public Health, Baltimore, since 1917, has been announced as the first recipient of the Borden Award, given by the American Institute of Nutrition. The 1944 prize was given to Dr. McCollum for his long years of pioneering research in nutrition.

Revista Sudamericana de Morfologia.—This journal (founded by Prof. Dr. A. E. Bianachi, Buenos Aires, Argentina; Prof. Dr. M. de Freitus Amorim, São Paulo, Brazil, and Prof. Dr. E. Herzog of the Instituto Pathologica, Concepción, Chile) publishes articles relating to anatomic (normal and pathologic), embryologic and anthropologic morphology in the Spanish and Portuguese languages, with English summaries. A volume of about 200 pages is published each year in two numbers (May and November) at the cost of $5.00 a volume ($3.00 a number). Publication began in 1943. The editor in chief is Prof. Dr. Andres E. Bianchi, Cordoba 827, Buenos Aires, Argentina.

Ewing Memorial Fund.—A fund has been established in memory of James Ewing which will be used to promote the teaching of human cancer to medical students and physicians. Contributions should be sent to the Memorial Hospital for the Treatment of Cancer and Allied Diseases, 444 East Sixty-Eighth Street, New York.